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SPRAY GUIDE

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REPORT AND ABSTRACTS OF THE 1959 MEETING OF THE NORTHEASTERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 19th Annual Meeting of the Northeastern Division of the American Phytopathological Society was held November 5 and 6, 1959, in the auditorium of Eastern States Farmers' Exchange, West Springfield, Massachusetts. Twenty-two scientific papers were presented. An evening session included a talk entitled "Experiences in Plant Pathology," by Dr. Charles Chupp, Emeritus Professor of Plant Pathology, Cornell University, and a showing of the Society-produced film "Design for Abundance." About 150 people attended the meeting.

Officers elected to serve during the next year are: *President*, J. E. Livingston; *Vice President*, D. H. Marsden; *Secretary-Treasurer*, R. H. Daines; *Councilor*, E. M. Stoddard.

D. H. Marsden, Secretary-Treasurer

Cross-inoculation of tomato and corn with Gibberella.—*Boothroyd, C. W.*—Green fruits of 4 tomato varieties and stalks of a single corn hybrid were inoculated with *Gibberella zeae* and *G. fujikuroi*. The isolates of *G. zeae* were from tomato fruits grown in a plastic greenhouse at Ithaca, N. Y., and from corn stalks collected in New Jersey, New York, and Pennsylvania; *G. fujikuroi* was isolated from corn stalks collected in New York. Inoculations were made by inserting toothpicks infested with *Gibberella* into green tomato fruits on the vines and into internodal stalk tissue of growing corn plants. A dark brown rot occurred in all tomato fruits inoculated with *G. zeae*, regardless of the isolates used. Macroconidia were formed in necrotic areas, and perithecia with abundant asci and ascospores developed in all tomato varieties about 3 weeks after inoculation. No rot developed in tomato fruits around picks infested with *G. fujikuroi* or around sterilized picks used as controls. All isolates of Gibberella rotted corn tissue, and *G. zeae* from tomato was as virulent in corn as were any of the isolates from corn. The New Jersey isolate of *G. zeae* from corn was the only one that produced perithecia on corn.

The pathogenicity of meadow nematodes as determined by the growth of strawberry plants in a commercial planting.—*Braun, Alvin J., and J. A. Keplinger.*—In a soil fumigation experiment conducted in a commercial strawberry planting, extremely low vigor was invariably associated with large populations of *Pratylenchus penetrans* within the roots, and, conversely, high vigor was always associated with low population levels. In nematocide treatment plots, where nematode counts in June of the year following planting ranged between 0 and 480 per gram of roots, plant vigor was superior to that in the untreated plots, where average counts ranged between 1860 and 4680. Prior to planting, samples taken from the untreated plots yielded 0–32 meadow nematodes per 100 cc of soil. Therefore, indications are that large populations can develop within the roots of susceptible crops from low initial numbers in the soil. The soil fungicide Terraclor (75% pentachloronitrobenzene), applied before planting at the rate of 400 lb./A., had no appreciable effect on the nematode population within the roots and no apparent effect on plant vigor.

Systemic disease control by 2-pyridinethiol-1-oxide and its derivatives.—*Cohen, Sylvan I., J. H. Reinhart and S. S. Ristich.*—2-pyridinethiol-1-oxide (Omadine) and several derivatives have been reported to exhibit limited systemic activity in cucumber and broadbean, moving principally downward. Root applications and stem immersion were ineffective. Derivatives have now been found that control foliar and root diseases of cucumber and tomato by upward and downward translocation after leaf, soil, and seed application. Localized movement was noted within leaves of cucumber and tomato by control of anthracnose and early blight. One application of 100 ppm of each of 11 derivatives to upper or lower leaf surface produced 10–96% disease control on the opposite surface. Control is consistently superior through lower surfaces. Applications to half a leaf surface reduced disease 38–82% on the untreated half. One mg of the zinc salt or disulfide applied to a cucumber leaf gave 77–91% systemic control on the leaf above and below. The water-insoluble zinc salt was almost as effective as the soluble disulfide. Soil drenches of 2 derivatives at 100 ppm also produced 50–82% control of cucumber anthracnose. Plants developing from cucumber seeds soaked in 2000 ppm of pseudothiourea derivative showed 42% reduction of anthracnose when the primary leaves were inoculated. Foliar spraying or seed-soaking of cucumber produced 70–80% control of Fusarium wilt.

Rusty spot of peach and its control in New Jersey.—*Daines, R. H., C. M. Haenseler, E. Brennan, and I. A. Leone.*—A tannish spot closely resembling old powdery mildew infections on peach fruits and named "Rusty Spot" by Sprague in 1955 has been observed in many New Jersey orchards during the past 4 years on Rio Oso Gem, Goldeneast, and Summer Queen varieties. Whereas Sprague found no evidence of fungi and decided against calling this a sterile fungus disease the authors have invariably found a scant but very obvious network of superficial hyphae close to the advancing edge of recently formed lesions. No spores or hyphae were found on the more central bald portion of older spots, where the dead corky cells were breaking away in small angular scaly sections. Fungicide trials on Goldeneast peaches in 1957 and 1958 showed that sulfur treatments provided good protection against Rusty Spot and that captan, glyodin, thiram, zineb, and ziram gave little or no control. These studies indicate that Rusty Spot as it occurs in New Jersey is probably caused by a pathogen that is able to kill the surface cells of the peach fruit but is unable to sporulate freely on the dead host cells. Similar phenomena are not infrequent in certain powdery mildews on hypersensitive varieties.

Relationships of population increase of Pratylenchus penetrans to vegetative growth of Wando peas.—*Dolliver, J. S., D. G. Clark, and W. F. Mai.*—Peas (*Pisum sativum* var. Wando) growing in 8-in. clay pots of steamed soil (½ sand, ½ loam, pH 6.5) with roots of orchard grass (*Dactylis glomerata*) infested with *Pratylenchus penetrans* were given treatments that affect plant growth. After 5 weeks, nematode numbers in each pea root system were determined. Conditions most favorable for plant growth were not best for nematode increase. For example, under controlled temperature and light conditions nematodes were fewest where plant growth was best. Greenhouse-grown plants from which pods were removed had larger root systems and fewer nematodes than did plants with pods. Treatments causing moderate restriction of plant growth resulted in higher nematode numbers than on untreated plants; on peas grown in sand with tap water, nematode numbers were double that on comparable plants given nutrients. However, treatments causing severe restriction of plant growth, e.g., defoliation or ringing of the stem, limited nematode numbers. The observed relationships could result from the production of inhibitory substances or from utilization by the growing plant of materials that are limiting factors for nematode increase within roots.

Effect of charge of ionized chemotherapeutants on their translocation through xylem.—*Edgington, L. V.,*

and A. E. Dimond.—The Helmholtz double layer at xylem walls gives them a negatively charged surface, which governs the distribution of organic ions moving through vessels. In tomato cuttings, organic ions were detected chemically and biologically. Compounds with anionic toxophores, e.g., sodium dodecyl benzene sulfonate, were systemic. Cationic toxophores, e.g., dimethyldidodecyl ammonium ion, were toxic only to the bases of stems. Sections of stems and discs of cellulose were exposed to solutions of test compounds. The quantities of compound were then analyzed chemically and biologically in terms of the amounts 1) removed from the solution, 2) eluted from the cellulosic substrate by washing, and 3) retained by adsorption on the substrate. Anionic materials were eluted by washing, but cationic radicals were not. Cationic toxophores are adsorbed on stems and discs by the negatively charged surface, where they remain fungitoxic. The anionic compounds move freely through the xylem into the leaf blades. The magnitude of the charge on a cationic chemotherapeutant affects the quantity of material adsorbed on xylem walls.

Relative infection potentials of rootstock and scion in increasing virus incidence in the deciduous tree fruit nursery.—*Gilmer, R. M., and K. D. Brase.*—Several economic viruses of deciduous tree fruits are transmitted in the nursery by infected propagating materials. The probability of virus infection of a nursery tree at propagation depends on 3 factors: virus incidence in the scions (V_s); virus incidence in the rootstocks (V_r); and effect of presence of the virus on scion union (K). Values of K depend on both the individual virus and the scion-rootstock combination. Total virus incidence (V_t) can be calculated from the formula $V_t = K(V_s + V_r - V_sV_r)$. When $V_s = 0$, V_t is determined solely by values of V_r and of K, so that, irrespective of the number of successive propagations, virus incidence remains at the level originally established in the initial propagation. Because of commercial propagating practices, when $V_s = 0$ but $V_r > 0$, the above formula becomes: ${}_nV_t = K(1-(1-V_r)^n)$, where n is the number of serial propagations. Thus, when infection is derived from rootstocks, an increment of virus infection is added with each successive propagation. In nursery certification programs, increased emphasis should be placed on virus-free rootstock procurement.

Cure of rose mosaic by heat.—*Holmes, F. O.*—Rooted cuttings from a mosaic plant of the variety of rose known as Grüss an Teplitz were held in 4-in. clay pots in a lighted incubator for periods up to 10 weeks. Light exposure was 16 hours daily. To prevent lowering of soil temperatures by excessive evaporation from the clay pots, each pot was enclosed in a plastic bag that was provided with bottom drainage; this permitted soil around the roots of plants to be maintained at temperatures only ½–1½°C below the air temperatures around the foliage. The soil temperatures were all within the range of 32½–36°C, although some plants were held first at lower temperatures and later at higher temperatures in this range with the purpose of acclimatizing them. Plants exposed to the incubator temperatures for 1, 2, and 3 weeks remained diseased, as did all of 8 unheated control plants. In contrast to this, plants held in the incubator 4, 5, 6, and 10 weeks subsequently developed only healthy-appearing foliage during an observation period of more than 9 months.

Salt injury to trees.—*Holmes, F. W.*—Sodium chloride (NaCl) and calcium chloride ($CaCl_2$) were applied from 1952 to 1958 (except 1953) to the sites of maples, oaks, 1 black birch, and 1 white pine. Applications were to the ground surface directly over roots weekly throughout the year, or only to adjacent road surfaces and only during a period of 15 consecutive weeks each winter. In both cases, the grass died by 1954, but no tree injury was noticed so soon. A sugar maple died after 186 weekly applications (1955–58) of 10 lb. NaCl each on the soil within a 12-foot radius. Chloride concentration in its leaves was 76 times, and in twigs 10 times that in check maples. Other maples, the birch, and the small pine died after 201 applications of NaCl over their roots, but a white oak so treated showed no symptoms. A red maple similarly receiving $CaCl_2$ bore stunted yellow foliage in 1958, but a black oak so treated appeared normal. Sugar maples adjacent to road surfaces salted with NaCl or with $CaCl_2$ 15 times at weekly intervals during each of 4 consecutive winters, showed a 3- to 6-fold increase of chlorides in leaves but no increase in twigs. These trees showed no symptoms of injury. Evidently the salt is accumulated in the leaves, and sloughed off with them each autumn.

Elm cottony tip blight.—*Kurtzman, R. H., Jr., and G. J. Stessel.*—A hitherto unobserved fungus disease appeared in late May, 1959, on 20% of the trees in elm plantations at Kingston, Rhode Island. Abundant white, cottony extramatrical mycelium development resulted in blighting of leaves and twigs. Only the current season's growth was affected, and the collapsed terminals remained hanging from older branches. Three to five days after the appearance of cottony growth, black sclerotia 2–7 mm in diameter formed in the mycelium. The disease was reproduced by inoculation of pure culture isolates into seedling elms under moist conditions. Above-normal moisture conditions favored initial disease development; heavy dew occurred each of the previous 12 mornings, and RH continuously exceeded 90% the 4 days before first observation. Since the fungus has so far failed to form other definitive structures, it can only be referred to the form genus *Sclerotium*.

Extraction of Ditylenchus dipsaci from organic soil and dried onion scales.—*Lewis, G. D.*—The screening, Baermann, and centrifugal-flotation techniques were compared for the extraction of *Ditylenchus dipsaci* from organic soil. The centrifugal-flotation technique yielded the highest number of nematodes and an almost complete separation from the soil particles. The Baermann technique yielded a fairly high number of nematodes, but many of them died in the funnel within 12 hours. The screening technique failed to separate many soil particles from the nematodes. A satisfactory method was developed for extracting large numbers of living preadult larvae of *D. dipsaci* from dried, infected onion scales. The dried scales were placed in a sieve suspended in a container of water. The suspension of nematodes and onion juice was removed and saved, and fresh water was added to the container hourly for 5 hours. The 5 suspensions were combined. The concentration of onion juice was reduced by a repeated process consisting of dilution followed by decantation after the nematodes had settled. This method allowed collection of ample numbers of *D. dipsaci* for use in nematocide assay tests.

Further cytological and cytochemical studies on the insect vector of aster yellows virus.—*Littau, Virginia C., and Karl Maramorosch.*—The discovery that the Eastern strain of aster yellows virus causes in its vector, *Macrosteles fascifrons*, cytological changes in the fat-body cells (a tendency toward stellate nuclei, obliteration of cell membranes, and sparse stringy cytoplasm) led to an investigation of various aspects of this relationship. The same cytological changes occurred whether the aster leafhoppers fed on diseased asters or on diseased carrots. So far, however, no such changes have been found associated with either of two other species of leafhopper that can acquire, but not transmit, the aster yellows virus. Most surprising was the finding that the celery-infecting strain of aster yellows virus had no cytological effect on the vector *M. fascifrons*, although it causes a very severe disease in the aster host. Examination of nuclear-volume–cytoplasmic-volume ratios in Carnoy-fixed tissue showed loss of cellular components in the viruliferous fat-body type, probably because the virus rendered these components more soluble in the fixative and other reagents. Efforts to identify and characterize the extractable material included enzymatic digestion, extraction of tissues on slides, comparison of various fixation methods, and histochemical tests.

The occurrence on Long Island, N. Y., of Micrutalis calva, a close relative of the vector of pseudo-curly-top virus.—*Maramorosch, Karl.*—In 1958 Simons and Coe reported from Florida the first vector of a virus among the *Membracidae* (treehoppers). This vector, a *Micrutalis* sp., transmits pseudo-curly-top virus. During the summer of 1959 numerous treehoppers were collected on a sandspit in Long Island Sound at Cold Spring Harbor, New York. These insects were identified by Louise M. Russell, Insect Identification and Parasite Introduction Research Branch, U. S. D. A., as *Micrutalis calva* (Say) sensu lato. They occurred along the beach on younger branches of the high-water shrub, *Iva frutescens* Man. subspecies *oraria*, but were not found on daturas or on other plants growing in the vicinity. According to Russell, the *M. calva* from Cold Spring Harbor is closely related to, but appears to be distinct from, the pseudo-curly-top vector of Florida. In greenhouse experiments the treehoppers were maintained for prolonged periods on *Iva* seedlings as well as on *Solanum nigrum*. Attempts failed to transmit *Ruga verrucosans*, the ordinary West Coast curly-top virus, by means of treehoppers to *Stellaria media* and tomato.

Bark patch grafts as a means of indexing for the stem-pitting virus.—*McCrum, R. C.*—The stem-pitting virus was found in a latent form in several apple varieties tested with bark patches of a variety sensitive to the stem-pitting virus. Elliptical patches of bark, cut from a Virginia Crab tree free of stem-pitting, were grafted to limbs of Cortland, Golden Delicious, and Red Delicious top-worked on 11 varieties of body stocks. Similar patches were also relocated as a control on the donor variety, Virginia Crab. The grafting was done on May 26 and June 3, 1959, and examined on September 10, 1959. No pitting was evident when Virginia Crab was grafted on Virginia Crab. Tissue produced under the bark patch was pitted on all 3 standard top-worked varieties tested. The pitting was typical in nature, but intensity varied with the various stock-scion combinations. It is still unknown whether the virus occurred originally only in the seedling root, only in the body stock variety, only in the scion variety, or in all of them. The bark-patch grafting technique seems to offer a quick method of indexing woody plants in which the virus disease results in abnormal wood and bark formation.

Inheritance of downy mildew resistance in broccoli.—*Natti, J. J., and J. D. Atkin.*—Resistance to downy mildew caused by *Peronospora parasitica* was obtained from a selection from the broccoli introduction P.I. 189028. Under greenhouse test conditions, sporulation was sparse on the cotyledons, but only localized spots devoid of sporulation developed on the true leaves of inoculated seedlings which originated from this selection. Under similar conditions, seedlings of the broccoli variety Waltham 29 were severely infected, and sporulation was heavy on both cotyledons and leaves. Crosses of lines known to be in a homozygous condition for resistance with completely susceptible plants of a selection of Waltham 29 broccoli produced resistant seedlings in all instances. Progeny tests of selfed plants from this F_1 generation resulted in segregations of resistant to susceptible seedlings in a 3:1 ratio, indicating that resistance was conditioned by a single dominant gene. A breeding program to develop a downy-mildew-resistant broccoli of the Waltham 29 type has progressed to the 5th backcross stage.

Cephalosporium associated with alfalfa root rot.—*Roberti, C.*—Field and laboratory observations during the last 2 years show that the crown-root rot complex is the major deterrent to alfalfa production in Rhode Island. Periodic pathogen isolations from young root lesions consistently yielded over 90% *Cephalosporium* colonies. A search of host indexes has failed to disclose reports of this fungus in alfalfa. Small, black specks or hairlike lesions start in the root epidermis and expand into sunken areas of cortex. Root hairs are killed and the primary root may die back. The fungus invades the xylem and pith, forming reddish-brown or brown streaks that may extend to the crown of young plants. Evidence indicates that this pathogen has a role in initiating the rot complex and may become present in 98% of plants in new stands.

Effect of Temperature on Post-Harvest Decay of Blueberry Varieties.—*Stretch, A. W., and B. H. Davis.*—Two early, 2 midseason, and 2 late blueberry varieties were stored 1 week in 1958 at 80, 55, 42, and 32°F. Humidity in storage was 80% at 80°F and about 50% at the other temperatures. Data on decay were taken prior to storage and after 1, 3, 5, and 7 days. Four pints of each variety at each temperature were examined and discarded after each evaluation in 2 separate tests. The principal molds found on decayed berries were *Alternaria* sp., *Botrytis* sp., and *Gloeosporium* sp. No significant differences in decay were noted after 7 days at 80°F in the early varieties Weymouth and Earliblue. Both varieties had about 77 and 43% molded berries in 2 tests. In the midseason varieties, Bluecrop showed 30 and 26%, and Rancocas 98 and 64% molded berries in 2 tests. Approximately 28% molded berries were noted in the late varieties Coville and Jersey in one test, and respectively 50 and 26% molded berries in a second test. At 55°F no differences were noted within the early and late varieties, but in the midseason group Bluecrop had significantly fewer molded berries than Rancocas. Progressively fewer molded berries were noted in all the varieties at 55, 42, and 32°F. The effect of pulp, veneer, and plastic containers on mold development, determined under similar storage conditions, showed no significant differences in rate of berry decay.

Necrotic ringspot, a new virus disease of cultivated blueberry.—*Varney, E. H., and L. C. Raniere.*—In 1955 a grower near Taunton Lakes, New Jersey, reported a virus-like disease on the Pemberton variety of cultivated highbush blueberry. Infected plants are stunted, show extensive twig dieback, and soon become unproductive. New leaves show chlorotic spots, rings, and line patterns. Chlorotic areas may become necrotic and drop out, giving the leaves a tattered appearance. Severely affected leaves are often reduced in size, and may be wavy and distorted as a result of the irregular expansion of the tissue. During the winters of 1956 and 1957, plants of the Pemberton, Stanley, and Burlington varieties were inoculated at budbreak in the greenhouse with chipbuds from diseased plants. Inoculated plants of all 3 varieties developed typical symptoms 2–4 weeks after inoculation. Check plants remained symptomless. The virus is readily transmitted mechanically from infected blueberry to *Nicotiana tabacum* and *N. rustica*, cucumber, cowpea, Bountiful and Pinto beans, *Physalis floridana*, *Chenopodium amaranticolor*, Cos lettuce, and Fire Chief petunia. Ring spots and line patterns characterize the disease in tobacco. Host range and vector studies are in progress.

The vitamin nutrition of Botrytis gladiolorum in relation to asexual reproduction.—*Wilcox, L. V., Jr.*—*Botrytis gladiolorum*, the causal agent of several types of diseases of gladioli, was grown on a liquid basal medium that contained salts, glucose, and selected amino acids. Vitamins were added singly and in various combinations, and the media were adjusted to pH 7.0 before being autoclaved. All tests were at 20°C. *B. gladiolorum* did not sporulate on vitamin-free medium or on thiamine-free medium containing the vitamins riboflavin, niacin, pantothenic acid, B_6, folic acid, B_{12}, biotin, C, and A. When these vitamins were used singly, sporulation occurred only with thiamine. Sporulation was greater when all vitamins were present than when either folic acid, biotin, or vitamin B_6 was omitted. Sporulation was fair when only thiamine was added, significantly greater when thiamine plus folic acid, biotin, or vitamin B_6 was added, and still greater when all 4 of these were present. Thus, *B. gladiolorum* is totally deficient for thiamine in relation to asexual reproduction and

is partially deficient for biotin, folic acid, and vitamin B_{12}. Data indicate that it is partially deficient for these 4 vitamins with respect to mycelial growth.

The effect of temperature on the protective action of selected fungitoxicants against snapdragon rust.—*Woodcock, W. P., and A. W. Dimock.*—Little information is available on the effect of temperature on the protective action of fungitoxicants against snapdragon rust caused by *Puccinia antirrhini*. Five fungitoxicants were uniformly applied as protective sprays to comparable snapdragon plants (*Antirrhinum majus* var. Jackpot), which when dry, were placed in artificially lighted chambers at 45, 55, or 65°F. After 7 hours, each plant was rapidly and uniformly inoculated with a standardized urediospore suspension in a 40° room, enclosed in a polyethylene bag, and returned to the temperature to which it had been adjusted. After 2 days, bags were removed and plants placed in the greenhouse. Primary pustules were counted 13 days after inoculation. The total number of pustules at all temperatures was: check 26,145, zineb 3, chloranil 32, captan 1134, copper 9200, and sulfur 10,890. The data indicate increased control with increasing temperature, the greatest differences being between 55 and 65°. The values for per cent control at 55° were: captan 94, copper 57, and sulfur 49; at 65°: captan 98, copper 73, and sulfur 72. Zineb and chloranil gave essentially complete protection at all temperatures. Under the uniform conditions used, one plant per treatment proved sufficient for the detection of fungitoxicant differences.

REPORT OF THE 51ST ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 51st Annual Meeting of the American Phytopathological Society was held in conjunction with the meetings of the American Institute of Biological Sciences on the campus of the Pennsylvania State University, College Station, Pennsylvania, August 29 through September 2, 1959. Attendance of Society members was estimated at about 500. The program consisted of a symposium, Training for the Future in Plant Pathology, sponsored by the Committee on Teaching of Plant Pathology; a Fungicide Colloquium of four papers; and 163 contributed papers on original research divided into sections on Diseases of Vegetable Crops, Diseases of Ornamentals, Physiology of Fungi, Fungicides and Antibiotics, Physiology of Diseased Plants, Diseases of Field Crops, Virology, Nematology, Diseases of Cereal Crops, Fungus Genetics and Inheritance of Disease Resistance, Fruit Diseases, and Soil Microbiology.

The Symposium on Training for the Future in Plant Pathology, under the chairmanship of Arthur Kelman, was as follows:

1. *Superior students and teachers in plant pathology.* John W. Oswald, University of California, Berkeley. Discussion: Webster H. Sill, Jr., Kansas State College.
2. *Improving introductory courses in plant pathology.* Russell Stevens, George Washington University. Discussion: M. B. Moore, University of Minnesota.
3. *Graduate courses, past, present, and future.* Glenn S. Pound, University of Wisconsin. Discussion: George C. Kent, Cornell University.
4. *Training plant pathologists as research scientists.* George L. McNew, Boyce Thompson Institute for Plant Research. Discussion: H. A. Rodenhiser, U. S. Department of Agriculture.

The Fungicide Colloquium, under the chairmanship of L. Gordon Utter, was as follows:

1. *Current status of Federal controls on fungicides.* J. A. Noone, National Agric. Chem. Assn.
2. *Present thinking on mode of action of fungicides.* Carroll Cox. Hugh Sisler, University of Maryland.
3. *Present status of use of foliar bactericides on vegetables.* Robert A. Conover, University of Florida.
4. *Industry presentations.*

The Council met on August 30 and 31 and September 2. General business meetings of the Society were held on August 31 and September 2. The annual banquet was held on Monday evening, August 31, in the Hetzel Union Ballroom. Over 400 attended the banquet, and many were unable to purchase tickets.

The Committee on Extension, Advisory Committee on Nematology, Committee on Regulatory Work and Foreign Plant Diseases, and many of the other committees of the Society held business meetings. Informal conferences were held on Diseases of Ornamental Plants, Diseases of Shade Trees, and Seed and Soil Treatment.

The Committee on Local Arrangements for the 51st Annual Meeting consisted of J. E. Livingston, Chairman, J. E. Wright, Co-chairman, H. B. Couch, H. C. Fink, L. R. Kneebone, James Tammen, and C. C. Wernham. The Chairman emphasized that there were many others who gave of their time and efforts in preparations for the meeting.

ORGANIZATION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY 1960

Council:

William C. Snyder, *President*, University of California
S. E. A. McCallan, *President-Elect*, Boyce Thompson Institute for Plant Research
William B. Hewitt, *Vice-President*, University of California
Glenn S. Pound, *Immediate Past President*, University of Wisconsin
George A. Zentmyer, *Secretary*, University of California
A. W. Dimock, *Treasurer and Business Manager of Phytopathology*, Cornell University
E. E. Wilson, *Editor-in-Chief*, University of California
C. S. Holton, *Councilor-at-Large*, State College of Washington
J. Ralph Shay, *Councilor-at-Large*, Purdue University
Thor Kommedahl, *Councilor of North Central Division*, University of Minnesota
E. M. Stoddard, *Councilor of Northeastern Division*, Connecticut Agricultural Experiment Station
B. R. Houston, *Councilor of Pacific Division*, University of California
L. O. Weaver, *Councilor of Potomac Division*, University of Maryland
Woodrow W. Hare, *Councilor of Southern Division*, Mississippi State College

Representatives:

A.A.A.S. Council: Carroll E. Cox (2 yrs., expires Dec. 31, 1960); H. A. Rodenhiser (2 yrs., expires Dec. 31, 1961)
A.I.B.S. Board of Directors: George L. McNew (4 yrs., expires Dec. 31, 1961)
Agricultural Research Institute, National Research Council: C. L. Lefebvre; E. L. LeClerg, Alternate
Division of Biology and Agriculture, National Research Council: Russell B. Stevens (3 yrs., expires June 30, 1961)
Food Protection Committee, Food and Nutrition Board, National Research Council: John R. McGrew
Board of Editors, American Journal of Botany: A. Frank Ross (3 yrs., expires Dec. 31, 1960)
American Type Culture Collection: John Stevenson; Ross W. Davidson, Alternate
Associates Food and Container Institute: M. A. Smith
Biological Stain Commission, Board of Trustees: Shirl O. Graham
American Standards Association Sectional Committee on Common Names for Pest Control Chemicals, K62: S. E. A. McCallan; W. D. McClellan, Alternate

American Grassland Council: Earle W. Hanson; John R. Hardison, Alternate

Editors:

Kenneth F. Baker (expires Dec. 31, 1960)
George W. Bruehl (expires Dec. 31, 1961)
E. C. Calavan (expires Dec. 31, 1961)
J. M. Daly (expires Dec. 31, 1962)
Stephen Diachun (expires Dec. 31, 1960)
Charles L. Fergus (expires Dec. 31, 1960)
Robert W. Fulton (expires Dec. 31, 1961)
Max W. Gardner (expires Dec. 31, 1961)
David Gottlieb (expires Dec. 31, 1960)
J. G. Horsfall (ex officio as Chairman of Committee on Reviews, expires Dec. 31, 1962)
William F. Mai (expires Dec. 31, 1962)
John H. Owen (expires Dec. 31, 1962)
Marr D. Simons (expires Dec. 31, 1960)
Hugh D. Sisler (expires Dec. 31, 1962)
Edwin B. Williams (expires Dec. 31, 1961)

Standing Committees:

Extension: Arden Sherf, Chairman; Gordon Brandes, M. P. Britton, Herbert G. Johnson, I. C. MacSwan, A. O. Paulus, E. G. Sharvelle, Harlan Smith, J. C. Wells

Finances and Investments: A. W. Dimock (ex officio), Chairman; A. E. Dimond, L. P. Miller, R. H. Wellman

History of Plant Pathology: J. A. Stevenson, Chairman; M. F. Barrus, H. P. Barss, M. W. Gardner, Karl Maramorosch

International Cooperation: F. L. Wellman, Chairman; A. Bitancourt, Carlos Garces-O, Ian Alexander Scott Gibson, John Grainger, J. G. Harrar, Lee Ling, Paul Neergaard, J. A. B. Nolla, Faustino T. Orillo, Rafael E. Pontis, C. S. Venkata Ram, Herman A. Rodenhiser, Luis Sequeira, A. J. Skolko, E. C. Stakman, John A. Stevenson, J. G. Ten Houten, Maurice F. Welsh, Neville H. White, B. H. Waite, Charalambos Zambettakis

Phytopathological Classics: L. C. Knorr, Chairman; Robert S. Dickey, J. C. Walker, E. E. Wilson (ex officio)

Phytopathological Monographs: C. S. Holton, Chairman; Helen Hart, A. C. Kelman, Harry E. Wheeler

Phytopathological News Page: K. W. Kreitlow, Chairman; Spencer H. Davis, D. E. Ellis, Shirl Graham, L. O. Weaver, Roy Wilcoxson, W. E. Sackston

Phytopathological Reviews: J. G. Horsfall, Chairman; J. S. Boyce, Harold J. Jensen, A. Frank Ross, W. H. Sill, Jr.

Placement: W. E. Cooper, Chairman; Eldon J. Cairns, J. J. Christensen, J. B. Kendrick, Sr., George Kent, H. Rex Thomas, Roy A. Young

Public Relations: P. R. Miller, Chairman; John B. Harry, E. B. Hayden, Paul Hoffman, C. E. Horner, J. B. Kendrick, Jr., T. H. King, John L. Weihing

Regulatory Work and Foreign Plant Diseases: G. L. Stout, Chairman; E. C. Blodgett, J. G. Dickson, J. P. Fulton, G. H. Hepting, R. P. Kahn, Gerald Thorne, W. H. Wheeler, Stephen Wilhelm

Seed and Plant Material Certification: Ralph M. Caldwell, Chairman; Wayne Bever, John Hardison, C. H. Kingsolver, C. M. Leach, W. C. Schnathorst, James Tammen, W. H. Wheeler

Special Committees for 1960:

Fungicide Colloquium: Saul Rich, Chairman; D. F. Crossan, J. B. Harry, W. A. Kreutzer, Curt Leben, J. E. Mitchell, P. D. Peterson, John Rowell, D. L. Stoddard

Industrial Relations: Paul Hoffman, Chairman; E. E. Feichtmeir, A. B. Groves, C. K. Krister, George L. McNew, N. C. Thornton, J. S. Tidd, W. J. Virgin, R. H. Wellman, Roy A. Young

Advisory Committee on Vernatology: J. N. Sasser, Chairman; Harold Jensen, M. B. Linford, W. F. Mai, W. J. Martin, D. J. Raski

Teaching of Plant Pathology: Arthur Kelman, Chairman; C. W. Boothroyd, I. W. Deep, D. W. French, J. W. Heuberger, J. E. Mitchell, J. H. Owen, G. K. Parris, L. F. Roth, Thomas Sproston, R. B. Stevens, Robert Zabel

Membership: Wm. B. Hewitt, Chairman; G. W. Bruehl, D. F. Crossan, A. W. Dimock (ex officio), Fred Frosheiser, W. J. Hooker, J. B. Lebeau, D. H. Palmiter, J. F. Schafer, J. B. Sinclair, E. B. Smalley, J. E. Thomas, N. N. Winstead

Utilization of Plant Pathologists and Facilities in National Emergencies: H. H. Thornberry, Chairman; A. L. Andersen, H. T. Cook, J. G. Leach, C. L. Lefebvre

Virus Type Culture Collection: H. H. McKinney, Chairman; R. W. Fulton, M. K. Brakke, T. J. Grant, R. L. Steere

Temporary Committees for 1960:

Advisory Committee on Collecting and Disseminating New Fungicide Data: L. G. Utter, Chairman; A. B. Groves, Fred H. Lewis, R. H. Wellman

Advisory Committee on Integration of Research on Soil-Borne Pathogens: W. C. Snyder, Chairman; L. F. Johnson, L. W. Koch, P. M. Simmonds, W. D. McClellan

Special Committee for Services to Manufacturing & Editing of Phytopathology: Glenn S. Pound, Chairman; A. W. Dimock, Helen Hart, A. Frank Ross, E. E. Wilson

Advisory Committee on Agricultural Meteorology: Paul E. Waggoner, Chairman; R. V. Bega, R. A. Hyre, Thor Kommedahl, S. M. Pady, L. H. Person, E. P. Van Arsdel, J. R. Wallin

Special Committee for Study of Possible Microbiological Center: W. C. Snyder, Chairman; W. A. Campbell, L. F. Johnson, J. A. Stevenson

Ad Hoc Committee to Study Committee Functions and Annual Meeting Programs: G. S. Pound, Chairman; Wm. B. Hewitt, S. E. A. McCallan, Ralph Shay, W. C. Snyder, George Zentmyer

Committee on Plant Disease Losses: Ralph Shay, Chairman; J. Cedric Carter, E. L. LeClerg, P. R. Miller, A. L. Smith, G. L. Stout, A. L. Taylor

REPORTS OF OFFICERS, COMMITTEES, AND REPRESENTATIVES

Report of the Secretary. The membership of the Society as of December 31, 1958, was 1,937. New member applications accepted were 187; of these, 71 were regular and 116 were student. There were 108 Life Members and 29 Emeritus Members. Since the preceding meeting the following members had passed away: Guy R. Bisby, Louise T. Dosdall, Hurley Fellows, R. J. Haskell, Clifford V. Kightlinger, Kenneth W. Neatby, Roy G. Pierce, Reiner Bonde.

WM. B. HEWITT

Report of the Treasurer-Business Manager. An analysis of the combined financial statement of the American Phytopathological Society and PHYTOPATHOLOGY will show that APS expenditures on regular funds exceeded regular income (dues) by $1,346.40 and that PHYTOPATHOLOGY expenditures exceeded PHYTOPATHOLOGY income by $4,015.35. The APS deficit may be partly accounted for by a share of costs of moving the business office, partly by the large amount of secretarial time devoted to handling matters related to the Golden Jubilee Celebration, and partly by increased committee expenditures. The PHYTOPATHOLOGY deficit is partly due to a $3,200 increase in cost of manufacturing PHYTOPATHOLOGY, partly to costs of moving the business office, and partly to costs of new investments. Because of a known backlog of manuscripts, the cost of manufacturing and distributing PHYTOPATHOLOGY will probably be as great next year. However, the normal increase in income from dues and subscriptions, the increase in income from investments, and the lack of major nonrecurring expenses of the 1958-59 year should help maintain a favorable balance.

A. W. DIMOCK

FINANCIAL STATEMENT
AMERICAN PHYTOPATHOLOGICAL SOCIETY
(INCLUDING PHYTOPATHOLOGY)

Fiscal Year Ended June 30, 1959

AMERICAN PHYTOPATHOLOGICAL SOCIETY

Receipts:

Balance on hand July 1, 1958		$30,473.35
Membership dues	$ 7,264.00	
NSF Grant G4605, supporting Ann. Volume	5,600.00	
NSF Grant G5742, supporting 50th Ann. Celebr.	3,400.00	
NSF Grant G5775, supporting Biblio. Rev. P. P.	8,000.00	
NSF Grant G8380, supporting Source Book	8,500.00	
Contr. Industry for 50th Ann. Celebr.	1,400.00	
Adv. sales 50th Ann. Volume	3,668.15	37,832.15
Total		$68,305.50

Expenditures:

Office of President	$ 13.00	
Office of Secretary	1,267.33	
Office of Treasurer	738.52	
Dues, AIBS, 1959	1,937.00	
Dues, American Grassland Council	10.00	
Donation, American Type Culture Collection	200.00	
Reprinting "Careers in Plant Pathology"	2,439.33	
Expenses, Repr. to Rutgers Inaugural	11.08	
Expenses, Pub. Relations Committee	712.16	
Expenses, NSF G5775 (Bibliog.)	2,775.79	
Expenses, Ann. Mtg., regular funds	1,281.38	
Expenses, 50th Ann., Ind. Contr. fund	6,241.89	
Expenses, 50th Ann., Rockefeller Grant	5,637.49	
Expenses, 50th Ann., NSF Grant G4605	16,437.79	
Expenses, 50th Ann., NSF Grant G5742	3,400.00	
Expenses, 50th Ann., APS Reserve fund	7,902.64	
Univ. of Wisc. Press, adv. sales	8,756.50	$59,762.50
Balance on hand June 30, 1959		$ 8,543.00

PHYTOPATHOLOGY

Receipts:

Balance on hand July 1, 1958		$27,656.27
Subscriptions and member-subscriptions	$23,382.81	
Advertisements	2,807.39	
Sale of back issues	1,107.37	
Reprints & excess illustrations	6,505.64	
Sustaining Associate Memberships	1,300.00	
Interest on Investments*	1,169.25	
Redemption of matured bonds	400.00	
Trans. from New Haven Savings Acct.	216.55	
Miscellaneous	233.46	$37,322.47
Total		$64,978.74

Expenditures:

Manufacture and Distribution of PHYTOPATHOLOGY	$27,854.04	
Cost of reprints	2,179.70	
Office of Business Manager	2,666.41	
Office of Editor-in-Chief	6,152.20	
Cost of new investments	1,182.85	
Refunds	204.75	
Office of Advertising Manager	186.04	
Director of Internal Revenue	911.83	$41,337.82
Balance on hand June 30, 1959		$23,640.92
TOTAL BALANCE APS & PHYTOPATHOLOGY		$32,183.92
Placed in savings and securities		29,290.55
Cash on hand June 30, 1959		$ 2,893.37

*Cash dividends only; accrued interest not included.

INVESTED FUNDS

Values as of June 30, 1959. Dividends and Interest for the Fiscal Year July 1, 1958 to June 30, 1959

SINKING FUND	Value 6/30/59	Earnings 1958–59
U. S. Savings Bonds, Ser. J	$ 1,875.00	$ 37.50
U. S. Savings Bond, Ser. F	23.22	.72
U. S. Savings Bond, Ser. E	378.00	3.00
Northw. Fed. Sav. & Loan Certif.	2,000.00	76.25
Lawn Sav. & Loan Certificate	8,000.00	320.00
Family Finance Corp., 90 sh.[a]	2,778.75	44.40
Westinghse. Elec. Corp., 22 sh.	2,079.00	44.00
Standard Oil (Ohio), 60 sh.	3,465.00	150.00
American Cyanamid, 100 sh.[b]	6,150.00	136.00
Johns Manville, 71 sh.	3,727.50	142.00
Amer. Tel. & Tel., 24 sh.[c]	1,926.00	
General Electric, 25 sh.[c]	2,006.25	
Pacific Lighting, 40 sh.[c]	1,960.00	
Boston Fund, 390 sh.[c]	7,386.60	46.80
Wellington Fund, 480 sh.[c]	7,555.20	52.80
	$ 51,310.52	$1,053.47

LYMAN MEMORIAL FUND

Westinghse. Elec. Corp., 35 sh.	$ 3,307.50	$ 70.00
Amer. Tel. & Tel., 66 sh.	5,296.50	198.00
	$ 8,604.00	$ 268.00

PHYTOPATHOLOGY CONTINGENCY FUND

Arlington-Fairfax Sav. & Loan	$ 8,150.89	$ 277.94
Arundel Federal Sav. and Loan	9,444.53	366.72
Southern Federal Sav. and Loan	10,089.09	393.66
Decatur Federal Sav. and Loan	9,041.40	308.32
Fidelity Federal Sav. & Loan	8,489.42	331.25
Standard Federal Sav. & Loan	10,028.95	391.31
Southwest Savings & Loan	8,307.46	324.14
Prudential Building Assn.	7,145.27	261.78
Tompkins County Savings Bank	2,075.00	75.00
Ithaca Savings Bank	2,090.00	90.00
First National Bank, Ithaca	1,068.75	68.75
	$ 75,930.76	$2,888.87
TOTAL INVESTED FUNDS	$135,845.28	$4,210.34

[a] 83 sh. purch. 4/59
[b] 20 sh. purch. 4/59
[c] Purchased 4/59

Report of the Auditing Committee. We have examined the financial record of The American Phytopathological Society and PHYTOPATHOLOGY for the fiscal year ending June 30, 1959. In our opinion all receipts, expenditures, and funds were properly and accurately recorded and in order.

G. C. KENT, Chairman
W. F. MAI
W. T. SCHROEDER

Report of the Editor-in-Chief. Material published in the July–December numbers of Volume 48 could not be covered in the 1958 annual report. These numbers contained 71 technical articles, 18 Phytopathological Notes, 83 abstracts (13 pages), 1 review article accepted under a program authorized by the Council in December, 1956, 3 book listings, the table of contents and index (18 pages), and 4½ pages of news. Some changes were made in the index: bold, medium, and thin type were used for the three grades of headings, and a special listing was provided for new species and varieties of pathogens. Six technical articles and 4 notes were rejected or withdrawn during the last six months of 1958.

Volume 49 will be the largest volume to date. As this report is written (July 1–10), 6 numbers have been published, the seventh is in press, and the eighth is in the paste-up stage. These numbers contain 532 pages. If the remaining 4 are each held to the customary number of pages (64), Volume 49 will contain about 772 pages. Volume 47, the largest previous number, contained 752 pages.

In the first 8 numbers of Volume 49 were 113 technical articles (460 pages), 28 notes (27½ pages), and 63 abstracts (11 pages). In addition, 15 pages were required for the annual report, 1½ pages for 8 book listings, and 1 page for an obituary. Pictures of Charter Members and Presidents of APS were printed on an extra signature (16 pages) in the April number. Three other numbers (May, June, and August) were also enlarged by 16 pages each.

In the first six months of 1959, 147 manuscripts were submitted. This is 37% greater than the number submitted in the first six months of 1958. As of July 1, 1959, 145 manuscripts were on hand. Ninety-seven of these had been accepted, and 58 of the 97 were in the galley stage, ready to be scheduled for publication. Thus, the number on hand would fill at least 12 numbers of the customary size. To avoid an undue delay in publication of this backlog, the size of future issues must be increased. We may be compelled, moreover, to establish 5 signatures as the usual number of pages per issue, thereby providing room for about 48 more technical articles and notes per volume.

Because of a heavy work load and production problems the printer fell behind in providing copy and in issuing PHYTOPATHOLOGY during the winter months. By late April, however, the normal flow of copy had been resumed and the numbers were again issued on schedule. Mr. John Ferguson III and his staff at Monumental are to be commended for the effective way in which they met these problems. For example, the average time between acceptance and publication of manuscripts in the May and June numbers was reduced to 151 days, comparing very favorably with the 160-day average for 1958. For the same period, however, the time between receipt and acceptance of manuscripts, was higher than that for 1958. To a considerable extent, this was due to the slowness of a number of authors in returning their revised manuscripts to the editorial office.

I wish to acknowledge the fine cooperation of authors in accepting manuscript changes. The editors and we in the editorial office suggest condensation or deletion only where we feel such can be done without impairing the value of the paper. One of our major responsibilities is to save space, thereby reducing cost. At times the deletion of words may seem to be "penny pinching," but with the cost of publishing scientific material at about 6 cents a word, a few words "pinched" puts us in the dollar class. Tables are more costly than either text or graphs. Moreover, tabular arrangement is not always the best for presenting results. Many types of data can be presented more effectively in the text or in a graph. When tables are used the data should be reduced to its simplest comprehensible form. How to prepare a table and when to use one is learned only by experience. Inexperienced authors tend to use too many complicated tables, leaving evaluation of the results largely to the reader.

I wish to thank the editors for careful evaluation of manuscripts. Though overworked most of the time, they performed this service cheerfully and effectively. I am indebted to the Business Manager for advice and help, to the officers of the Society for encouragement and support, to Mr. Kelvin Deming for efficiently performing the tasks of both a copy and a production editor, and to Mrs. Betty Shaw, who as Secretary has kept the office running smoothly.

E. E. WILSON

Report of the Advertising Manager. Seventy-five pages of ads and news were printed in PHYTOPATHOLOGY during the fiscal year ending June 30, 1959. There were 52.5 pages of paid ads and 8.5 pages of news; the remaining 14 pages were free ads for the Society, *Phytopathological Classics*, *Entoma*, and *Biological Abstracts*. The American Phytopathological Society has received free advertising in *Entoma* in exchange for *Entoma* ads in PHYTOPATHOLOGY.

Receipts and disbursements are reported by the Business Manager.

I am grateful to Louis Brown, Monumental Printing Company, A. W. Dimock, Business Manager, and E. E. Wilson, Editor-in-Chief, for their help in matters pertaining to advertising in PHYTOPATHOLOGY.

D. A. ROBERTS

Report of the APS Representative, Board of Editors, American Journal of Botany. The representative on the Board of Editors received no communications from the editorial staff of the American Journal of Botany.

A. F. ROSS

Report of the APS Representative, AIBS Board of Directors. The Governing Board of AIBS held two meetings during the past year. The first was at the termination of the meetings in Bloomington, Indiana, on August 28, 1958, under the presidency of Dr. Fenn. The second, under the presidency of phytopathologist James B. Dickson, was held in Washington, D. C., on April 28 and 29, 1959. Your Society's representative was most favorably impressed with the purposefulness of this body, scope and magnitude of its various operations, and the general efficiency of the Institute's management by the Executive Secretary, Hiden T. Cox.

The Institute operates on a close budget that is kept balanced by exceptionally alert management. The total income from members in 1958 was $15,721.50. Dues from affiliates and other sources of income for general operations brought the total income to $31,836.04. Total expenses of the AIBS offices were $53,500.83, but the deficit was more than offset by the recovery of $47,240.44 in overhead charges on certain grants for sponsored projects operated by the Institute.

The total amount of money received from grants in 1958 was $372,382.99, which was added to $99,526.01 of funds carried over from 1957. This, with income from publications, gave a total of $481,412.16 for operating all projects. Among the major projects operated in 1958 were translation of Russian journals ($96,888 from NSF), publication of an English edition of Russian Soil Science ($36,575 from NSF), conducting a Visiting Lectures Bureau for small liberal arts colleges ($24,843.60 from NSF), the Biological Sciences Register ($22,415.66 from NSF), International Conferences in Botany and Zoology ($20,227.34), development of an experimental TV film ($47,800), studies on curriculum in science by high school teachers and college professors to be done at the University of Colorado ($143,200 from NIH), and development of a series of moving pictures for teaching an integrated program in biology (Ford Foundation grant of $172,191).

Substantial progress is being made in consolidating publication services and in improving the Institute's publications under a qualified and experienced publications manager. The AIBS Bulletin still operates at a deficit, but the Quarterly Review of Biology and the Symposia Proceedings are operating on a sound financial structure.

In view of the financial situation and the excellent services being performed by AIBS, your representative strongly endorsed and urged the removal of the $1,000 ceiling per

society, a move that APS had already voluntarily adopted. This move, opposed primarily by the Science Teachers and the Ecological Society, was debated at length at the spring meeting, and the general consensus was that the rate of $1.00 per member was actually lower than would be desired and that larger groups should not receive favored treatment in the future.

Upon recommendation of Counsel, the AIBS articles of incorporation were amended so as to obtain complete tax exemption under the District of Columbia and the Federal Government. Your representative vigorously opposed the wording of the amendment that provided that AIBS would operate under the Internal Revenue Code of 1954 and any *subsequent* amendments thereto. It seemed unreasonable to accept as part of our operating procedure a specific article that could be implemented by future executive decrees of government administrators whose names and objectives are unknown to us. Your representative was also opposed to the following provision: "To the extent allowed by the regulations of the various government departments and agencies the work of the corporation (i.e. AIBS) shall be made available to the public through periodic publications." It was our opinion that this clause should be rewritten so that it would clearly apply only to regulations regarding contract work for the government, not to all operations of AIBS. Your representative was also concerned over possible misinterpretation or abuse of a provision specifying that AIBS should not carry on propaganda or attempt to influence legislation. Political propaganda is not an objective of AIBS, but a published fact can be construed as propaganda if it conflicts with one's own ideas. All these provisions were voted in because Counsel and the Executive Secretary urged no changes in the preliminary draft since wording of the document already had been approved by federal and district officials.

I urge most strongly that APS continue to pay $1 per member, irrespective of the total amount. We are receiving about $5 per person in services, either direct or indirect.

GEORGE L. MCNEW

Report of the APS Representative, AAAS Council. As representative of the Society on the Council of the AAAS, I voted for the election of Chauncey D. Leake as President-Elect, and for Bentley Glass and Margaret Mead as members of the Board of Directors.

Unfortunately I was not able to attend the annual meeting of the Association in Washington this year. To me, one of the important occurrences during the year for AAAS was the meeting of its new standing Committee on Social Aspects of Science, on May 30–June 1, 1958. I did not attend, of course, but have a report.

The report says "It is therefore understandable that the past year's efforts have been aimed more at a resolution of these difficulties (a defining problem) than at the performance of specific programs of action.

"It is *not* the purpose of the Committee so to define these issues, facts and interpretations as to become an advocate of particular public policies or courses of action, or to become an agency of propaganda. It *is* the purpose of this committee to recommend to the Board various ways in which scientists may participate in deliberations regarding the use of scientific knowledge. This does not imply that the AAAS should never take a stand or recommend a specific policy or program. The committee opinion does reflect the belief that many current problems of science and society have moral and political aspects which belong properly in the domain of public decision; that informed decision is hindered by failure to distinguish moral and political from scientific aspects; that decision is further complicated by the existence of varying or conflicting schools of thought on the interpretation of available scientific evidence, each with its partisans and propagandists; and that, in such situations, AAAS can best meet its responsibilities, not by adding a partisan voice, but by attempting to separate the issues, identify the schools of thought, and explain and review the interpretations of evidence. The consideration of radiation hazard illustrates the point. In these situations, AAAS may serve an authoritative—not an authoritarian—function."

The new Vice-President for Section G is F. W. Went, of the Missouri Botanical Garden, and for Section O, R. E. Hodgson, Agricultural Research Service, Beltsville, Maryland.

JAMES G. HORSFALL

Report of the APS Representative, Division of Biology and Agriculture, National Research Council. The National Research Council met as a single body for the bulk of the time allotted to its annual meeting, March 19–21. Four general topics were explored, first in plenary session, then in sectional discussion meetings, and again in plenary session: Means and Patterns for the Support of Scientific Research; The Nature and Conduct of Fellowship Programs in Science; The Place of Behavioral Sciences in the National Research Council; and U. S. Science in Foreign Aid. We can expect a summary report of the deliberations, but it would be infeasible to attempt any further details at this point.

Obviously, a large number of the many and varied activities of the Academy and of the Research Council affect, more or less directly, the affairs of plant pathologists. They do not, at the same time, seem to be matters that can be readily reported explicitly in this report. I refer to the activities of the Fellowship Office; the U. S. Committee of the International Union of Biological Sciences; Documentation Conference; Conference on Scientific Manpower; studies of renewable natural resources, of research programs in oceanography, etc.

The Division of Biology and Agriculture is organized to meet the needs of the moment, so to speak, and therefore reflects the changing interests and emphases of the groups whom it most immediately serves. This is not to say that the Division is entirely at the mercy of outside pressures, but rather to admit that whatever is vigorously pushed is inclined to loom rather larger in the organizational and budgetary pattern of the Division. At the moment, the agricultural and nutritional programs and the work on laboratory animal resources comprise much of the active element in the Division; the Handbook of Biological Data somewhat reduced emphasis; and some once-active committees and boards now largely inactive or no longer existent.

During the "fiscal" year April 1958–March 1959, the Biology Council and Committee on Educational Policies were discontinued, not through any lack of recognized value in many quarters, but simply because funds for their continuation were not forthcoming. The boards and committees in the general area of food and agriculture continued at a normal pace, as did the program on laboratory animals. The Handbook office has reduced its staff and curtailed some of its projected volumes, but otherwise continues much as always and in the past year completed its treatment of fungicides.

News items since March, contained in a very valuable informal monthly news sheet sent from the Division office, include: activities of the Committee on Bio-Astronautics, a very large and far-reaching group; a gift to the NAS-NRC of funds to construct a new Hall of the Life Sciences; resumption of the work on the High School Sourcebook in Biology; and a grant of $50,000 to the Agricultural Board for the specific use of its Policy Committee. Very recently there was a meeting looking toward reestablishment of the Committee on Educational Policies in a somewhat altered form and initial steps toward establishing a board to survey the manifold implications of the widespread use of pesticides in field and forest.

It has been my experience through several years as a staff member with the National Research Council, and two since that time as a most interested bystander, that astonishingly few American scientists have even the haziest notion of what the Academy-Research Council is, what it does, and how much more it could do with the support of individual scientists. Because it is basically neither an operational

organization nor a fund-granting group, and because its role vis-a-vis the Federal Government is almost universally misunderstood, few have any appreciation of its unique potentialities and function. By this I do not mean "better," or "larger," or "more profound"—I mean just that: unique.

The annual meeting of the Division—as distinct from the annual meeting of the National Research Council—was brief, lasting only a few hours. It was clear, or so it seemed to me, that most members of the Division representing affiliated societies (except those few who, like myself, were intimately aware of Division activities through personal and continued contact) were nearly as ignorant of what the NRC stands for as is the general scientific public. Yet if the time and effort of these men, the unpaid committee and board members, and of the paid staff, are to yield dividends, the societies must make use of the Division in whatever ways may prove fruitful.

I would urge that the Council consider carefully which of its problems might be passed along to the Division with recommendations for action. No miracles will result, but I think we would be pleasantly surprised to learn what a proper question, intelligently framed and diligently pushed, might yield through the NRC channels. At some risk of oversimplifying a most highly complicated situation, I would suggest that if a question is of a research nature it must be handled by the individual pathologist in his own laboratory and field plots; if it is an operational problem it properly belongs with the demonstratedly productive staff of the AIBS; if it entails matters of policy, particularly as they may impinge on other areas of science, it should be laid at the doorstep of the NRC through the Division.

As a specific example of this pattern of operation, I call to mind a matter considered briefly at the recent Division meeting: the whole problem of experimental populations, breeding and seeding stocks, natural areas, etc., in the plant sciences. This is not something to be considered in the individual laboratory. It is not as yet, by any means, something to be started on a large operational scale. The pros and cons need to be thrashed out on the broadest possible base, and action taken only after deliberate considerations. Is there a problem here for plant scientists? For the special area of plant pathology? What disciplines are involved? What is the range of material to be considered? What are the costs? What are the obstacles to this solution and to that? I would by no means limit Council consideration of how best I and the NRC can serve the Society to this one point, but it serves as one example. What, if anything, would the Council like to see done in this area? What is my charge as Society representative?

If the Council does not use the Division to the maximum, I can function in my capacity as representative only as an individual, and somewhat feebly at that.

RUSSELL B. STEVENS

Report of the APS Representative, Agricultural Research Institute. The Seventh Annual Meeting of the Agricultural Research Institute was held in Washington, D. C., October 13 and 14, 1958, but little transpired at this session regarding plant pests.

At its meeting in July the Agricultural Pest Committee adopted a policy that it would serve entirely as a stimulating group to develop interest and to encourage activity in every line of activity represented by the various subcommittees. The committee eventually plans to turn the whole project of developing information on losses and on biological control of diseases over to specialists, for the committee felt it was not desirable to stay in the field indefinitely.

On October 20 and 21 the Committee on Agricultural Pests held for the first time a joint meeting of its six subcommittees dealing with insects, plant diseases, nematodes, vertebrate pests, weeds, and biological control of soil-borne plant pathogens. The purpose of the conference was to devise methods for, and stimulate interest in, accurate appraisal of losses caused by agricultural pests. Two reports pertaining to crop losses came to our attention during the year: (1) Some Principles of Survey Methods and Procedures for Determining Samples, by E. L. LeClerg, and (2) Progress Report on New Techniques for Plant Disease Surveys and for Appraisal of Losses, by Paul R. Miller. Brief reports were also prepared by subcommittees, indicating that the Agricultural Pests Committee was active.

The Eighth Annual Meeting of ARI is scheduled for October 12 and 13, 1959, in Washington, D. C.

E. L. LECLERG
C. L. LEFEBVRE

Report of the APS Representative, Food Protection Committee, Food and Nutrition Board, NAS-NRC. The Eighth Annual Meeting of the Liaison Panel of the Food Protection Committee, held in Washington, D. C., December 11–12, 1958, was devoted to review papers and panel discussion on food packaging materials, their composition and uses. The report of the Subcommittee on Food Technology appears as NAS-NRC Publication 645. None of the material presented was directly related to plant pathology.

One recent publication of the Food Protection Committee that may be of interest to plant pathologists is a statement resulting from the 1956 symposium on insignificant levels of chemical additions in food. It appeared in the Food Drug Cosmetic Law Journal, July, 1958, and concludes: "It is recognized that, as the result of use of chemicals in food production and processing, substances may occur in foods in amounts so small as to be inconsequential in relation to public health. In such instances, regulatory action is not required."

J. R. MCGREW

Report of the APS Representative, American Type Culture Collection. Total receipts by the Collection for 1958 were $82,889.76, and expenditures were $71,220.99. Of this amount, $60,289.28 was obtained from the sale of cultures, $17,756.86 from grants, and the balance from miscellaneous sources. The number of cultures (9231) sold in 1958 was the largest except one (1949) in the Collection's history. Income from this source has been augmented ($3350) pleasantly by the fees, at $50 per culture, for the deposit of cultures in connection with patent applications. Total worth of the corporation now exceeds $100,000, of which about $40,000 is in cash and negotiable securities and the remainder represents equity in the building housing the Collection. The balance due on the building has now been reduced to about $16,000.

The 6th edition of the ATCC catalog of cultures was issued in October. Copies are being sold at a dollar each, a procedure that helps defray the costs of preparation and publication. Listed as available for sale are 4350 strains of organisms distributed among 1847 species. Added during 1958 were 331 strains. The second edition of the Catalog and Registry of the Plant Viruses, also issued late in 1958, is available on request. It is noted that APS contributed $200 to the cost of publishing this catalog. Manuscript for the revised catalog of animal viruses has been completed and is under review by interested workers. To be published during 1959, it will list 140 strains, in contrast to the 52 strains in the 1950 edition.

Dr. Clark, Associate Curator, received a new grant of $10,580 from the National Institutes of Health to continue his work on bacteriophages. The same Institution also approved a grant of $5000, effective September 1, 1958, for expanding and improving the services of the ATCC Viral and Ricksettsial Registry. The Rockefeller Foundation provided $749.86 to enable the project to get under way.

The Curator, Dr. Freeman Weiss, prepared a demonstration covering the work of the Collection as part of the exhibits of the APS at its 50th anniversary meeting at Bloomington, Indiana, in August. Some 300 persons visited the exhibit.

The annual meeting of the ATCC Board of Trustees was held on February 21, 1959, at Washington, D. C. Reports

of the Curator and Associate Curator were received and approved. A proposed budget totaling $78,342 was approved for 1959. A retirement system for ATCC employees was adopted, and the necessary legal steps taken to put it into action. Attention was given to improving ATCC services along various lines but in particular in cooperation with the National Institutes of Health in the field of maintenance of virus and tissue cultures. A new Executive Committee was appointed, consisting of R. D. Coghill, G. W. Irving, and C. L. Lefebvre (Chairman). The Board recorded its appreciation of the services of H. H. McKinney, representative of APS on the Board for many years and Chairman of the Executive Committee for the past two years. The Board elected John A. Stevenson to succeed Mr. McKinney, with R. W. Davidson as alternate.

JOHN A. STEVENSON

Report of the APS Representative, American Standards Association Sectional Committee on Common Names for Pest Control Chemicals, K62. The number of common names officially accepted was increased by 9 during the past year, bringing the total to 18, as follows:

Common name	Class	Chemical name
chlorbenside	miticide	*p*-chlorobenzyl *p*-chlorophenyl sulfide
dalapon	herbicide	2,2-dichloropropionic acid
dicapthon	insecticide	*O*-2-chloro-4-nitrophenyl *O,O*-dimethyl phosphorothioate
dimethoate	insecticide	*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate
diphacinone	rodenticide	2-diphenylacetyl-1,3-indandione
diuron	herbicide	3-(3,4-dichlorophenyl)-1,1-dimethylurea
dodine	fungicide	*n*-dodecylguanidine acetate
erbon	herbicide	2-(2,4,5-trichlorophenoxy)ethyl 2,2-dichloropropionate
ethion	acaracide insecticide	*O,O,O',O'*-tetraethyl S,S'-methylene bisphosphorodithioate
fenuron	herbicide	3-phenyl-1,1-dimethylurea
monuron	herbicide	3-(*p*-chlorophenyl)-1,1-dimethylurea
neburon	herbicide	1-*n*-butyl-3-(3,4-dichlorophenyl)-1-methylurea
ovex	miticide	*p*-chlorophenyl *p*-chlorobenzenesulfonate
phorate	insecticide	*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate
phosphamidon	insecticide	2-chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate
ronnel	insecticide	*O,O*-dimethyl *O*-(2,4,5-trichorophenyl) phosphorothioate
silvex	herbicide	2-(2,4,5-trichlorophenoxy)propionic acid
zoalene	anti-coccidial drug	3,5-dinitro-*o*-toluamide

Standard common names for fungicides coined by the Interdepartmental Committee of the USDA in cooperation with the Society and prior to the formation of the above Committee K62 are: captan, chloranil, dichlone, ferbam, glyodin, maneb, nabam, thiram, zineb, and ziram. Chemical names for these are given in PHYTOPATHOLOGY 45: 295-302. 1955. The new fungicide dodine is also known under the trade name Cyprex.

A meeting was held at the ASA headquarters in New York on November 12, 1958, to consider various current problems.

A Third Plenary Session of the International Committee on Common Names for Pesticides, ISO/TC81, is planned in London, October 6–9, 1959. At the end of 1958 there were 12 participating member nations: Canada, France, Germany, India, Italy, Mexico, Netherlands, Poland, South Africa, Sweden, United Kingdom, and U.S.A.; and 21 observing members: Australia, Austria, Belgium, Brazil, Bulgaria, Chile, Czechoslovakia, Denmark, Greece, Hungary, Ireland, Israel, Japan, New Zealand, Pakistan, Portugal, Romania, Spain, Switzerland, Turkey, and Jugoslavia. This committee is concerned primarily with (a) principles for selection of common names for pesticides, (b) procedure for submission of common names to ISO/TC81, (c) proposals for common names, and (d) means of promoting national adoption of ISO-recommended names. A large number of proposed common names are under consideration.

Continued representation by APS on ASA Sectional Committee K62 is recommended. S. E. A. MCCALLAN

W. D. MCCLELLAN, Alternate

Report of the APS Representative, American Grassland Council. The American Grassland Council (formerly Joint Committee on Grassland Farming) held its annual business meeting November 28, 1958, in Chicago, Illinois. I was unable to attend this meeting but submitted a written report as follows: "The American Phytopathological Society held its 50th Anniversary Meeting August 24–28 at Bloomington, Indiana. These meetings were held in conjunction with those of the American Institute of Biological Sciences. The major part of the program was devoted to symposia with invitational papers being presented by specialists from all over the world. Since major emphasis was placed on the symposium program, presentation of research papers was discouraged in order for those in attendance to hear the symposia speakers and participate in discussions.

"The exhibit hall at the meetings was devoted principally to plant pathology research. All aspects of pathological research, including research on diseases of grassland crops, were covered in the many excellent exhibits displayed.

"Interest in diseases of forage grasses and legumes continues to mount, as evidenced by the number of papers presented at Divisional meetings of the Society. Interest is also evidenced through requests forage pathologists receive for preparation of articles for farm journals, books, and reference volumes such as encyclopedias."

On January 27–30, 1959, the American Grassland Council held a joint meeting with the American Society of Range Management at Tulsa, Oklahoma. This meeting was in keeping with the practice of holding joint meetings in cooperation with societies represented in the American Grassland Council.

The American Grassland Council decided to issue a quarterly publication reviewing pertinent grassland activities in the United States. The publication is not intended to compete with recognized existing journals but would review recent progress in the various fields and present these not only to members of the council but to people interested in grassland agriculture in general. A publication board made up of representatives from various subject-matter areas or fields of interest within the Council was activated. As representative of APS, I was approached and accepted the assignment of serving on the publication board for the field of phytopathology. The first number of *Grassland Progress* should be issued during July, 1959.

Each society or organization affiliated with the American Grassland Council was asked to designate an alternate representative to the Council. In compliance with this request, Dr. Earle W. Hanson, Plant Pathology Division, University of Wisconsin, Madison, was so designated.

K. W. KREITLOW

Report of the APS Representative, Biological Stain Commission. Nothing to report.

SHIRL O. GRAHAM

Report of the APS Representative, Research and Development Associates, Food and Container Institute. Your representative has been in contact with the Research and Development Associates, Food and Container Institute for the Armed Forces, throughout the past year. The Activities Reports were received and studied. An extremely interesting and informative report on the Preservation of Food by Radiation appears in the Activities Report for April, 1959.

The annual meeting of the Research and Development Associates was held in Washington, D. C., March 31 and April 1 and 2. One important session was that of Packaging and Materials Handling, conducted by the Quartermaster Food and Container Institute staff. This program included a talk on Irradiation to Protect Storage Items.

Colonel James P. Littlejohn (QMC), USA, assumed command of the Quartermaster Food and Container Institute for the Armed Forces in February, 1959. He succeeds Colonel Ervin L. Keener, who was reassigned to other duty.

Working through a contract with the Quartermaster's Food Preservation Project, your representative and his associates have continued studies in this laboratory on the control of postharvest diseases of fresh fruits and vegetables by means of gamma radiation. During the past year, studies were continued to determine factors influencing the survival following gamma radiation of representative fungi *in vitro* and *in vivo*. In addition, certain causes for the failure of commercial-lot irradiations were investigated. One basic factor emerging from these studies was that a fast close irradiation rate was more effective than a slow rate in curtailing rate of decay.

M. A. SMITH

Report of the APS Committee on Extension. Our Committee met formally on August 25, 1958, at Bloomington, Indiana, and discussed possible projects for 1959. A subcommittee was appointed to plan a program for an extension workers' section meeting at the 1959 annual meeting, to be held in Pennsylvania.

The Committee sponsored an informal meeting of all extension pathologists at Bloomington. This two-hour meeting was well attended—32 pathologists from many states. This offered an opportunity for extension workers to become better acquainted and for newer men to meet a number of the veterans of 25–30 years. Informal discussions centered on 1) the new plant disease slide sets, which were exhibited. 2) the problem and need for continuing representation at the Federal level of our extension project, 3) exhibition of a new Wisconsin movie on garden diseases, and 4) discussion of topics desired for the 1959 extension session.

A letter from our Committee on behalf of all extension pathologists was drafted and submitted to Mr. C. M. Ferguson, administrator of the Federal Extension Service in Washington. This was in regard to the need for a Federal extension plant pathologist to replace the late Dr. R. J. Haskell. Other similar letters were solicited from our Society president, college department heads, and other pathologists. U.S.D.A. officials should now be cognizant of the strong backing of our membership for extension support at the Federal level. We hope this can be fulfilled when funds become available again.

The main project of our Committee this year was the collection, selection and preparation of 2×2 color transparencies of plant diseases. Seven sets, devoted to 1) cereal and forage, 2) tree fruit, 3) small fruit, 4) cotton and sugar beet, 5) tomato and potato, 6) muck crops, and 7) miscellaneous vegetable diseases, were completed and offered for sale nationally at a modest price. These were high-quality, well-chosen slides submitted by numerous cooperating APS members. Excellent publicity in national, professional and nonprofessional agricultural magazines resulted in a complete sell-out by June 15 of 626 sets, for a total of $4,752.09. This was a nonprofit, nonexpense venture that appeared highly worthwhile as an APS Extension Committee service.

An effort is being made to organize extension-worker groups within the five sections of our Society in order to permit closer cooperation on projects and events of mutual interest to extension pathologists.

ARDEN F. SHERF, Chairman

Report of the Committee on International Cooperation. It is believed by several members of this Committee that a definite stand should be taken on the question of its most effective form of organization. It was suggested at the meeting of the Committee in August, 1958, that, perhaps because of difficulties encountered by foreign members in travel, it might be best to confine full memberships to North American residents, with others in a "corresponding member" status.

Some letters, and later personal discussions, have shown that foreign members are a little bit sensitive about this suggestion. Some members believe they have in the Committee a special and strong relationship, and they want to do even more than at present. It seems that they would feel that being a corresponding member would not be a happy solution. To tell the truth, it is the Chairman's belief that his fellow Committee members want him to do more than he has this year and make the group a more important adjunct to the Society.

It is therefore proposed that the Committee continue to operate as at present constituted, carrying on as much as it can by correspondence. This is difficult because it is a fairly good-sized Committee and communication takes some time. This will have to be recognized as part of the Committee chairman's responsibility, and he will have to write more letters. It may be at times that major decisions requiring rapid action will depend on the chairman. It is hoped that much can be done by members at annual meetings, as in the past. When it comes to most of the decisions the chairman will poll his members by letter and act according to the results. PHYTOPATHOLOGY is designated as "An International Journal" on its masthead. The Committee should be so handled as to be a source of ever stronger international aid to the Society.

The Committee has started compilation of an international directory of plant pathologists. It is not ready to report much progress on this now, but it is developing. In another year the list should be ready for publication.

There was a proposal, welcomed by this Committee, that we be prepared to assist in whatever manner possible connected with the 5th International Congress of Plant Protection. Correspondence was carried on toward the end of learning where the Congress would be held, hoping for its coming to the U.S.A. It was found that the place of meeting seems to depend on what country can offer best subsidy and facilities. The U.S., it seems, does not come into this category at present.

The Committee is in full accord with a proposal that as many as possible of the countries, especially those with fewer mycological and phytopathological personnel, bring up to date and publish plant disease lists. There has been good response to this, but it will require some time and correspondence to go further on it.

Another well received proposal consists of Committee assistance to Dr. Lee Ling, F.A.O., Rome, in his international plant-pathological program. It will require a further canvassing of suggestions. Some have already given tentative suggestions and reactions.

A badly needed List of International Phytopathological Organizations is being prepared. Dr. Paul Neergaard has started this and suggests that with the list should be given a brief account of the activities of each organization.

A project of international cooperation intent is now in progress under Dr. M. F. Welsh. He is at work on a worldwide use of terminology for plant virus diseases. A proposal has also been made that a list of general plant disease

names in the world be prepared, either for publication or at least for deposit in a few leading libraries of the world.

It is believed by members of the Committee that correspondence with Committee members needs much greater attention. It has been suggested that a small special subsidy be granted by the APS to this Committee for hiring a part-time secretary, and mailing and stationery costs. This would be used as follows: 1) Informational letters sent at least four times a year to each Committee member. 2) Compilation of such lists as are suggested by the Committee or the APS. 3) Manuscript work related to the Committee's activities. 4) Help in literature search and keeping up to date, foreign plant disease occurrences, foreign society meetings, changes in foreign plant pathologist personnel, and such matters.

FREDERICK L. WELLMAN, Chairman

Report of the Committee on Finances and Investments. During the past season our investment portfolio has been examined by 3 independent investment counselors: C. E. Treman, of the Tompkins County Trust Company, Ithaca, N. Y.; Studley, Schupert & Co., of Philadelphia, Penna.; and J. S. Barr & Co., of Ithaca, N. Y. Though differing in detail, all advised a shift away from the high proportion in Insured Savings and Loan Shares to an increase in high-grade common stocks, and preferably toward some form of professional management. Investment in reliable managed investment funds appeared to be the most reasonable move in the latter direction. To effect a change in balance we have increased our investments in common stocks and have made appreciable purchases of shares of 2 outstanding managed funds—the Boston Fund and the Wellington Fund. These purchases have been made entirely from the rather large cash balances maintained in anticipation of expenses of the Golden Jubilee Celebration. The change in balance of investments has thus been achieved by increasing our total investments rather than by realignment of previous holdings. The proportion of our holdings in securities has in this way been increased from 15% to somewhat over 35% of our total invested funds. A summary of operations during the 1958-59 fiscal year follows:

	Original investment	Value June 30, 1958	Earnings 1958-59
Cash or equivalent:			
Ins. Sav. & Loan Passbk.*		$70,697.01	$2655.12
Ins. Sav. & Loan Certif.	$10,000.00	10,000.00	396.25
Savings Accts., new*		5,233.75	233.75
U. S. Savings Bonds	2,193.50	2,276.22	41.22
		$88,206.98	$3,326.34
Securities:			
Common stocks			
Previous holdings	$12,081.06	$23,011.63	$ 743.20
Purchased April, 1959	9,994.13	9,684.87	41.20
Investment funds			
Purchased April, 1959	14,992.20	14,941.80	99.60
		$47,638.30	$ 884.00
Totals		$135,845.28	$4210.34

Total invested funds were valued at $135,845.28 as of June 30, 1959, and earned $4,210.34 in the year ending June 30, 1959. The yield of only 3.2% reflects the recent stock purchases. On previous investments, the yield was 4.1%, compared with 4.2% the preceding year.

Transactions for the past fiscal year:

1. American Tel. & Tel. common stock split 3 for 1, increasing our holdings (including new purchase) from 22 to 90 shares.

2. Redeemed matured Series F bonds worth $400; reinvested $375 in $500 maturity value Series E bond.

*Irregular deposits and withdrawals preclude realistic statement of original investment.

3. Interest and dividends allowed to accrue in savings accounts.

4. Purchased April, 1959:

390 sh. Boston Fund at $19.18	$7,480.20
480 sh. Wellington Fund at $15.65	7,512.00
20 sh. Amer. Cyanamid at 57½	1,150.00
8 sh. Amer. Tel. & Tel. at 256¾	2,054.00
25 sh. General Electric at 83¾	2,093.75
40 sh. Pacific Lighting at 50¾	2,030.00
83 sh. Family Finance at 32⅛	2,666.38
	$24,986.33

5. Transferred higher proportion of cash-on-hand from check to interest (savings) accounts.

A. W. DIMOCK, Chairman

Report of the Standing Committee on History of Plant Pathology. Among interesting additions during the year to the historical file were some of the records of the Fiftieth Anniversary meeting of APS. Other such items, including tape recordings of the invitation papers given during the meetings and the moving picture film "Design for Abundance," will be received for deposit at a later date. Other accessions have included additional photographs of APS members, presented by Dr. Marvin D. Whitehead, and a series of papers from Dr. M. F. Barrus dealing with APS activities during the first world war. All items in the collection are available to interested workers at any time.

JOHN A. STEVENSON, *Chairman*

Report of the Committee on Phytopathological Classics. Titles in the PHYTOPATHOLOGICAL CLASSICS series were displayed at a booth during the Society's Jubilee Celebration, in Bloomington. Featured was the newly issued tenth volume: *Del Mal del Segno*, a translation from the writings of the pioneer Italian pathologist Bassi de Lodi. It is hoped that by pricing PC 10 at production cost, new readers will be introduced to the series and to the satisfactions that derive from knowing the origins, development, and flowering of phytopathological thought.

A list of titles in the PC series that are currently available appeared in the June, 1958, issue of PHYTOPATHOLOGY.

The report of the Treasurer of PHYTOPATHOLOGICAL CLASSICS, Dr. Robert S. Dickey, for the fiscal year July 1, 1958, through June 30, 1959, is as follows:

Receipts:				
Cash balance, July 1, 1958			$1,827.98	
Received during fiscal year 1958-59:				
From sale of CLASSICS		$333.00		
Originals	$301.50			
Reprints	31.50			
From accounts receivable		10.50		
Originals	9.00			
Reprints	1.50		343.50	$2,171.48
Disbursements:				
Cost of printing CLASSIC No. 10		$1127.18		
Bank charges		2.75		
Refunds		2.42		
Stationery and supplies		23.24		
Postage		18.39	1173.98	
Cash balance on June 30, 1958			$ 997.50	$2,171.48

The number of copies of PHYTOPATHOLOGICAL CLASSICS sold during the fiscal year was 295, having a total value of $343.50. Numbers of CLASSICS on hand at Ithaca, New York, and at Lake Alfred, Florida, on June 30, 1959, were as follows: No. 1, 423; No. 2, 453; No. 3, 0; No. 4, 49; No. 5, 279; No. 6, 329; No. 7, 254; No. 8, 443; No. 9, 642; and No. 10, 860.

L. C. KNORR, *Chairman*

Report of the Committee on Phytopathological Monographs. The Committee has continued its efforts to obtain manuscripts of diseases suitable for monographic treatment. With various changes the Committee still has promises of 13 manuscripts. No manuscripts solicited by the Committee have yet been received. Several manuscripts have been received and some rejected; others are awaiting revision by the Committee.

Three authors have requested financial assistance and the Council has set up provisions for handling such requests. One author, as a result of change in positions, has requested transfer of his monograph assignment.

G. C. KENT, *chairman*

Report of the Committee on Phytopathological News Page. During 1959, alternate half and full pages of news have or will be published in each issue of the journal except March. The full page of news scheduled for March had to be reduced to one-half page, because of advertising commitments. With occasional exceptions, news items have continued to reach the editor's desk in sufficient volume to fulfill our space obligations. More unsolicited items are being sent in by individuals in addition to those forwarded by members of the Standing Committee for preparation of News. It is also encouraging to note that items are being voluntarily contributed by foreign members. It is hoped that this trend will continue and that we will be able to provide our membership with news items concerning the field of phytopathology on a world-wide basis.

For able assistance in preparing the news page in 1959, the Editor wishes to acknowledge his appreciation to the members of the Standing Committee: L. O. Weaver, Shirl O. Graham, D. E. Ellis, S. H. Davis, Roy D. Wilcoxson, and E. J. Hawn.

K. W. KREITLOW, Editor

Report of the Committee on Phytopathological Reviews. It seems that the activities of the Committee now fall into two rather distinct categories, as follows:

Solicitation and processing of review articles.—Alarm expressed at the Council meeting of a year ago seems to have been unfounded. There has certainly not been a flood of review articles swamping the journal to the detriment of research reports. Indeed, the Committee has not yet received even one of the several (seven, I think it is) articles solicited last year and reported to the Council at that time. As Chairman I take full responsibility for not having pressed these matters upon the authors with greater vigor, but in any event I would hope that most, if not all, will be forthcoming within the next 12 months. Certainly, it now appears that, without a very great promotional effort, it would be impossible to secure enough material to maintain a separate reviews journal in plant pathology, and that for the foreseeable future such items as we do receive can be accommodated within the present arrangement.

One manuscript, from Dr. Gäumann, has been published; a second, offered by Professor Farkas, in Budapest, should be forthcoming in due time.

Preparation of a bibliography of review articles.—It will be recalled that the first effort of the Committee after its formation was to survey those outlets for reviews in plant pathology that were most conspicuous and to assemble a file of reviews "in press" or "in progress" in the hope that we might thus avoid duplication. This file has, as far as possible, been kept up to date through the past two years.

As a direct outgrowth of this program, the Committee sought and obtained a grant of $8,000 from the National Science Foundation for the preparation of an initial bibliography of reviews in plant pathology, with the intention of publishing this in PHYTOPATHOLOGY as a special issue, to be followed by annual supplements appearing as an integral part of a regular issue. Details of this plan, and Council action pertinent thereto, will be found in the minutes of previous meetings of that body.

Work has been going on, largely in the library of the Department of Agriculture, for about 9 months. Within the limitations attending any such prediction, it rather looks as if we shall have about 1500 items over a period of 10 years (1949–59). Of the original grant, we have thus far spent $3,403 for salaries and $177.79 for materials. Since both the bibliographer and the typist have been somewhat more productive than I originally estimated, I believe we can budget sufficient of the remaining funds to complete the ten-year survey, prepare the cards for photographing, and purchase the plates needed for printing within the initial estimates and have nearly enough left to prepare the first annual supplement, i.e., the 1960 material. My estimate for this second job is about $900, of which I hope to find $600 from the present grant. I should, therefore, like to request that the Council appropriate $300 toward this end. If this can be done, I will request the National Science Foundation to allow an extension in time sufficient to permit preparation of the first supplement. With tangible evidence of Society support, I think there will be no problem on this issue, particularly when I emphasize to them the greater desirability of moving an additional year into the future rather than using the money to push the survey back beyond the present 1949 starting date (i.e., reviews dated from 1949 on).

I should make it clear to the Council, and then to the Foundation, that I am not simply suggesting the extension of the present bibliography for an additional year, to result in an 11-year survey appearing in 1961. Quite the contrary. I propose that the ten-year survey be terminated as of the December, 1959, journals, prepared, printed, and distributed as soon as practical after that date. The additional year, provided the Council and Foundation act favorably on my petition, would be the first of the planned annual supplements and would give us a good measure of the time, techniques, and funds required to prepare these on a continuing basis.

May I suggest that I continue in my present capacity as director of this particular project until its final completion, even though I will have served my term as Chairman of the Committee and member of the Editorial Board? Any attempt to shift project officers at this point might lead to unnecessary complications with respect to the grant, and would surely delay completion of the bibliography.

I should like to express my appreciation to the members of the Committee for their generous support and advice during the past several years, and to the Council for its courteous attention to my recommendations.

RUSSELL B. STEVENS, Chairman

Report of the Committee on Placement. The Committee has processed a total of 67 applications of plant pathologists during the past year. A study of the date the applications were filed shows that 22 were filed over 2 years, and 15 from 1 to 2 years ago, and 30 during the past year. Of these, 0, 9, and 6, respectively, had their applications removed from the active files (because they had accepted new employment), and 3, 2, and 1, respectively, had decided to remain at their present position. In addition, 2, 0, and 4, respectively, accepted new positions but desired to keep their applications in an active status. There are 46 applications now in the active file.

The above study indicates the difficulty of placing individuals whose records have been on file for an extended time. Part of this may be due to these applicants' training, productivity, recommendations, etc.; employer reluctance to consider these may be the out-of-date records. To correct this, we have suggested to some of the applicants that they submit new applications, thereby presenting a more complete record to the employer. Two have already done so.

Applications were sent to 50 employers. The number of applications sent to each ranged from 2 to 13, averaging 4 per position. The total number of applicant-employer contacts made was 209. Most of these positions have been filled, though not often through contacts made by the Placement Committee.

Persons interested in Federal positions should fill out Standard Form 57 and mail it to the Executive Secretary, Board of U. S. Civil Service Examiners, Sixth Floor, Ad-

ministration Building, U. S. Department of Agriculture, Washington, D. C. There is, on most Land-Grant College campuses, an Agricultural Research Service Contact Representative who can supply additional information or forms.

The activity of the Placement Committee is more effective if administrators with positions to be filled notify the Placement Committee Chairman. This eliminates an exchange of letters before the Committee submits the records of applicants for the position.

It is the responsibility of the applicants to keep the Committee informed of changes in address or employment status. If there is no reply after 3 attempts to contact the applicant, his application is placed in the inactive files. All records and files are currently up-to-date.

W. E. COOPER, *Chairman*

Report of the Committee on Public Relations. This year our Committee revised the brochure "Careers in Plant Pathology." Ninety thousand copies were sent to the 48 State Counselors for further distribution. The remaining 10,000 copies were retained by the Chairman of this Committee. Other work has continued along the lines initiated 5 years ago: publicizing some of the outstanding plant-disease research papers. Selected material appearing in PHYTOPATHOLOGY has been used in advance through the cooperation of the Editor-in-Chief, who made galleys available before publication. Plans for extensive news coverage (by press, radio, and television) at the Pennsylvania State University meetings are being coordinated with those of AIBS. Press releases for about 25 contributed papers that seem to have publicity value will be issued at the time of presentation at University Park.

PAUL R. MILLER, *Chairman*

Report of the Committee on Regulatory Work and Foreign Plant Diseases. The Committee met in two sessions during the Golden Anniversary meeting of the Society at Bloomington, Indiana, in August, 1958. As in the previous year, it had again invited the members of the Committee on Seed and Plant Material Certification and of the Advisory Committee on Nematology to meet jointly with it to consider some problems of mutual interest. It also had invited some other people who had a work interest in subjects on the agenda and who might be helpful in the deliberations or who had reports to make to the Committee. Some of the members of the other two committees and several of the other people accepted the invitation and attended.

The agenda included (1) European rhododendron rust; (2) potato rot nematode; (3) indexing of imported plants and plant-propagative materials for detection of viruses; and (4) seed-borne plant pathogens.

Six members, E. C. Blodgett, J. G. Dickson, E. G. Rex, G. L. Stout, G. Thorne, and W. H. Wheeler, of the Committee on Regulatory Work and Foreign Plant Diseases, attended the first session, on Sunday afternoon, August 24. The Committee on Seed and Plant Material Certification was represented at this session by W. H. Wheeler, who was a member of both committees; the Advisory Committee on Nematology was represented by H. J. Jensen. The group was pleased to have, also, at this session, A. D. Baker, Canadian Department of Agriculture, Ottawa, Canada; J. L. Creech, U. S. Department of Agriculture Plant Introduction Station, Glenn Dale, Maryland; H. M. Darling, University of Wisconsin; A. M. Finley, University of Idaho; R. P. Kahn, U.S.D.A. Plant Quarantine Division, Glenn Dale, Maryland; and H. F. Winters, U.S.D.A. Plant Introduction Section, Beltsville, Maryland.

The second session, on Monday evening, August 25, was attended by E. C. Blodgett, J. G. Dickson, W. C. Snyder, G. L. Stout, and W. H. Wheeler of the Committee on Regulatory Work and Foreign Plant Diseases; by K. F. Baker, D. J. Hagedorn, W. C. Schnathorst, and W. H. Wheeler, of the Committee on Seed and Plant Material Certification; and by phytopathologists E. W. Hanson, University of Wisconsin, R. P. Kahn, U.S.D.A., and Paul Neergaard, Danish Government Plant Protection Service, Copenhagen, Denmark. Dr. Neergaard has had a special interest in seed-borne plant pathogens, as Chairman of the Plant Diseases Committee of the International Seed Testing Association, and this session was arranged so that he could meet with the group for a mutual exchange of ideas and information.

The items on the agenda for the Bloomington sessions were considered and acted upon as follows:

1) *European rhododendron rust* (first session).—Committee member Wheeler reported on this rust, *Chrysomyxa ledi* (Alb. & Schw.) d By. var. *rhododendri* (DC.) Savile, for the U.S.D.A. Plant Quarantine Division. Consideration of the subject was a follow-up on the problem concerning this rust that had been referred to the Committee by Director E. P. Reagan, of the Plant Quarantine Division, a year earlier. The problem had been considered by the Committee at the Palo Alto meetings, and an opinion was given at that time by the Committee. Its opinion was that the biologically sound course to pursue was to continue to contain the infection where it existed in the State of Washington; to eradicate the infection if possible; and, in the meantime, to continue the federal post-entry quarantine requirements then existing on imported rhododendrons because of this rust fungus (see Report of the Committee on Regulatory Work and Foreign Plant Diseases, Item 4, Rhododendron—Spruce Rust Problem, PHYTOPATHOLOGY 49: 171-172, 1959).

Wheeler brought the Committee up to date on events concerning the problem. He explained the work that had been done toward eradication of the rust, and told about the biologic and economic difficulties encountered. It had been difficult to find and destroy all of the infections and to prevent new infections; but efforts were being continued and fungicidal treatments were being applied. It was pointed out that more survey work is needed to determine the distribution of this rust.

Following a discussion of Wheeler's report the Committee expressed the opinion that future action on this problem appeared to be a matter of administration within the U. S. Department of Agriculture.

2) *Potato rot nematode* (first session).—This nematode, *Ditylenchus destructor* Thorne, was the subject of regulatory attention in recent months after it had been found infecting dahlia roots in Oregon. Committee member Gerald Thorne gave a brief history of it as it attacks potatoes in Idaho and Wisconsin. Damage to the potato crop apparently varies with conditions. H. J. Jensen, in discussing the nematode in dahlia in Oregon, said it is very difficult to detect it in dahlia roots because symptoms are very difficult to recognize. It was pointed out that this nematode is known in many other host plants and that it, in fact, is reported to be widespread in this country and in various other parts of the world. It was suggested that the Committee go on record as pointing out to the authorities of the various states that the potato rot nematode is actually more than just a potato pest. At the same time, caution was suggested against hasty quarantine action by the states in view of the reported rather large distribution of this nematode in the United States.

Following a rather thorough and lengthy discussion, the Committee prepared the following statement:

"The Committee on Regulatory Work and Foreign Plant Diseases of the American Phytopathological Society, in a regular meeting on August 24, 1958, devoted a considerable amount of time to hearing statements on the potato rot nematode, *Ditylenchus destructor* Thorne, by several workers, including Gerald Thorne, University of Wisconsin, who described the species; H. M. Darling, University of Wisconsin; A. D. Baker, Canada Department of Agriculture; H. J. Jensen, Oregon State College; and A. M. Finley, University of Idaho.

"A summary of the statements and ensuing discussion on the host range, distribution, and control of the potato rot nematode, briefly, is as follows. *Ditylenchus destructor* Thorne should be regarded as a polyphagus species, not

confined to potatoes. It occurs without doubt in bulbous iris as it does in potatoes. Morphologically this nematode appears to be the same in dahlia as in potato. It also is found in carrot, sugar beet, onion, dandelion, and many other plants, as well as in numerous fungi. In view of this, the APS Committee on Regulatory Work and Foreign Plant Diseases suggests that anyone who might discuss or consider regulatory action against *Ditylenchus destructor* Thorne should take cognizance of its wide host range.

"It was the consensus of the group that the Chairman of the APS Committee on Regulatory Work and Foreign Plant Diseases should address this communication to the APS Secretary, with the request that he transmit it to the National Plant Board, scheduled to meet at the Imperial Hotel, Portland, Oregon, September 16, 17, and 18, 1958."

(Chairman Stout of the APS Committee transmitted this communication to APS Secretary Wm. B. Hewitt, who transmitted it to Chairman F. Herbert Gates, of the National Plant Board. Also, Chairman Stout attended the Portland meeting of the NPB, where he discussed the communication and the action of the Committee.)

3) *Indexing of imported plants and plant-propagative materials for detection of viruses* (first session).—The indexing of certain imported plants and plant-propagative materials for the detection of plant viruses has been a subject of previous discussions and recommendations by the Committee and by fruit tree research virologists and other interested persons, including the Interregional Technical Committee for Stone Fruit Virus Research (now known as the Interregional Coordinating Committee); and the Society, at the Atlanta meetings in December, 1955, recommended the appropriation of funds by Congress to make possible the appointment of a plant pathologist to conduct the indexing. (PHYTOPATHOLOGY 44: 722-723 and 723-724, 1954; 46: 352-353, 1956; and 49: 170-172, 1959.)

Director E. P. Reagan, of the federal Plant Quarantine Division, reported to the Committee, prior to the Bloomington meetings, that the Division had employed Dr. Robert P. Kahn, on October 1, 1957, to carry on the work.

Dr. Kahn gave a progress report on the work to the Committee at the Bloomington meetings. The program was inaugurated in October, 1957, by the Nursery Stock Section of the Plant Quarantine Division, in cooperation with the New Crops Research Branch of the Crops Research Division and the Entomology Research Division, with emphasis on virus diseases of plants under quarantine at the U. S. Plant Introduction Station at Glenn Dale, Maryland. Essentially the program consists of indexing by insect, sap, dodder, or graft transmission according to standardized procedures, but it likewise includes the detection of viruses by serological methods and the conduct of basic research to support this program. Living plant material introduced from abroad is being examined to detect the presence of plant diseases that may be new, harmful, or a threat to the agriculture of the United States, and to devise measures and techniques that would allow valuable plant material to enter in a sanitary condition. The indexing is necessary because many plant diseases, especially virus diseases, cannot be detected by the usual visual methods of inspection.

At present the major emphasis is on *Prunus*, *Solanum*, and *Ipomoea*, but some consideration will also be given to other kinds of plants, including ornamentals and new crops, and to the detection of viruses in interceptions made by plant quarantine inspectors. Dr. Kahn hopes to have about 25 *Prunus* introductions completely indexed in 1959, another 25 in 1960, and 50–100 in succeeding years. He had indexed 40 or more different potato importations and about 6 of *Ipomoea*.

4) *Seed-borne plant diseases and the problem of laboratory examination of imported seeds for plant pathogens* (first and second sessions).—The hazards of introducing plant diseases by their causal agents carried in or on imported seed have been the subject of previous discussions. The Committee and the Society had recommended that the U. S. Department of Agriculture consider laboratory examination of imported seeds for the detection of seed-borne pathogens (PHYTOPATHOLOGY 47: 319, 1957; 48: 117-118, 1958; 49: 170-172, 1959). Director E. P. Reagan, federal Plant Quarantine Division, reported to the Committee, prior to the Bloomington Meetings, that his Division had discussed the matter with the Seed Branch of the U. S. Agricultural Marketing Service in an effort to formulate a possible plan of action. Problems involved were the location of laboratory facilities and funds for the work.

At the first session of the Committee at the Bloomington meetings, Willis H. Wheeler reported further for the Division, saying that both the ARS and the AMS had been considering the matter and understood the need for some kind of work. Exploratory tests are needed to determine what pathogens are coming into the United States on imported seeds, which would provide information that would be helpful in developing and financing a program. The Committee authorized Edgar G. Rex to explore the possible means of accomplishing the exploratory tests.

The Committee reviewed a letter that APS Secretary Hewitt had received from Marion Viccars, of the Marion Viccars Seed Testing Laboratory (commercial), Montgomery, Alabama, and had referred to the Committee for consideration. The letter discussed various problems and needs in the matter of testing seeds for plant pathogens and urged the Society to give consideration to them.

At its second session, the Committee had a very interesting exchange of information with Dr. Paul Neergaard, of Denmark, who opened a discussion on seed testing for plant pathogens on an international basis. There is need for a uniform method of seed testing for pathogens, and many countries are not prepared to test seeds to satisfy the plant quarantine requirements of the countries of destination. As a part of the solution to the seed certification problem, Dr. Neergaard emphasized the need for similar seed examination methods if similar results are to be obtained. He outlined some of the seed testing procedures in Europe and reported that an effort is being made to work out a standardized procedure. He suggested a survey to compile a list of areas in the world where clean seed could be grown, and indicated that the European and Mediterranean Plant Protection Organization had shown interest in such a survey. He expressed an interest in communicating with specialists who could give information on diseases that should be considered of quarantine significance and who might be able to furnish color slides of such diseases. The group expressed its appreciation to Dr. Neergaard for the unusual opportunity of meeting with him.

GILBERT L. STOUT, Chairman

Report of the Committee on Seed and Plant Material Certification. No formal meeting of the Committee held during the Anniversary Meeting. K. F. Baker requested that he be relieved of responsibility as Chairman, and the present Chairman was notified of his appointment some time later.

Only one specialist has stated an intent to prepare a short monograph for the series on diseases caused by seed-borne pathogens. W. C. Schnathorst has indicated that he will prepare a monograph entitled "Seed-borne diseases of cotton."

A meeting is to be held during the 1959 annual meeting, at which we hope to reiterate the intent of the Committee and assume active responsibility again.

WAYNE M. BEVER, Chairman

Report of the Committee on Fungicide Colloquium. The Fungicide Colloquium was deferred at the last annual meeting because of the special program arranged for the 50th Anniversary Program of APS. Recommendations, however, were made for the Colloquium to be resumed at the current annual meeting. These recommendations were favorably approved by the Council.

Various difficulties were encountered with respect to developing the current program, among which was obtaining acceptances for the invitation papers. In all cases refusal was due to expected nonattendance by the person

concerned. The finalized program is reported in the introduction to this 51st Annual Report.

The Committee recommends that, because of its past recognition, interest, and importance, the Colloquium be included on the program of the annual meeting in 1960. It also requests that the Secretary schedule the Colloquium on the program and retain this scheduling, since abstracts of papers are not required of those invited to participate. The Committee will provide the program for the Colloquium in time for the printing of the Annual Meeting Program. Unless the Council has contrary opinions, it is also recommended that the current Committee be continued for another year.

L. Gordon Utter, *Chairman*

Report of the Committee on Industrial Relations. During 1959 there were no requests for assistance from the Society, and no organized program by the Industrial Relations Committee. It was generally felt not advisable to initiate programs requiring solicitation of funds from industry this year because of our active program last year in conjunction with the Golden Jubilee Celebration.

There was some feeling that the Society and the Committee could profit by regular rotation of the chairmanship and membership of the Committee. Inclusion of university and industry members on the Committee seems desirable to provide a balance of viewpoints. It was also suggested that the Committee size be reduced to facilitate operation.

E. F. Feichtmeir, *Chairman*

Report of the Advisory Committee on Nematology. At one of the informal discussion periods of the nematologists at Bloomington, Indiana, the question arose as to the advisability of the nematologists forming their own society. A number of nematologists and plant pathologists interested in the science briefly gave their views. Some very convincing ideas were set forth both in favor of and against a separate organization. Some expressed the view that to take the whole step now would be unwise, and recommended that sectional status within the Society would perhaps meet our needs and yet keep us in close contact with the parent society. After considerable discussion, the group, by almost unanimous vote, authorized the Advisory Committee on Nematology to consult informally with various Council members and other colleagues, and find out what could be done to meet the needs of the nematologists. The Committee was further requested to prepare a report to be presented at an announced meeting to be held during the APS meetings at Penn. State in 1959. Following is the report of this Committee:

1) The Committee has determined that the Council members of APS recognize our problems and are anxious to cooperate in working out a solution.

2) The Council would definitely like the nematologists to remain in APS, and is willing to do whatever is possible to meet our needs within the framework of APS.

3) The Council encouraged us not to consider a separate society or journal at this time, reminding us of the financial difficulty the virologists have encountered in establishing a separate journal.

4) Several Council members recommended sectional status, but pointed out that they could not see any advantages we would gain that we do not now have, except some degree of autonomy.

5) Dr. Pound outlined to the Committee the steps necessary to petition for a formal section organization.

6) The Council recommended that we make use of machinery already set up; that is, Advisory Committee on Nematology, to work with the other officers of APS in arranging papers, special discussion periods, and other matters of common interest to the nematologists.

In consideration of these recommendations by the Council and as a result of our own study, the Advisory Committee would like to make the following recommendations for your consideration:

1) The Committee feels that nematology will eventually be identified as a separate science, and that the formation of a society of nematology is inevitable. At present, however, the Committee feels that a separate society would be unwise because of the small number of full-time nematologists and the fact that our present needs can be met within the framework of APS and yet we can enjoy the benefit to be derived from being closely allied with APS. It was concluded that a proposal for a separate society at a more appropriate time would have the blessings of all concerned.

2) The Committee does not recommend petitioning for sectional status at this time, since there are no advantages to be gained that we do not already have.

3) The Committee cannot see any advantage of a separate journal at present, since publishing has not become a problem. *Phytopathology*, *Plant Disease Reporter*, *Helm. Soc. of Wash.*, and *Nematologica* all accept worthwhile papers in nematology.

4) The Committee further recommends that arrangements for papers at annual meetings, special discussion periods, symposia, etc., be made through the Advisory Committee on Nematology, and that this committee be made a permanent committee rather than a special committee.

5) The Committee recommends that the above committee make a continuing study of the needs of nematologists, and bring recommendations to the group when it is deemed wise.

J. N. Sasser, *Chairman*

Report of the Committee on Membership. The various States and Canada were apportioned among the Committee members, as in recent years. Each member then contacted a likely person on the various campuses or large industrial units within his area, who was asked to canvass persons interested in plant pathology. The major activity was in the fall, so as to contact new graduate students. We believe that the special drive last year, in connection with preparation of the new Directory, rounded up many of the potential members still at large.

S. E. A. McCallan, *Chairman*

Report of the Committee on Teaching Plant Pathology. The first meeting of the Committee was held on August 28, 1958, at Bloomington, Indiana. The following proposals were considered: 1) organization of subcommittees, 2) preparation of a proposal for development of a sourcebook of laboratory exercises to be submitted to the National Science Foundation, and 3) compilation of information on teachers of plant pathology and subject matter currently available at individual schools that might be useful for preparing the sourcebook of laboratory exercises.

The following persons were appointed to serve as chairmen of regional subcommittees: Ira Deep, Western Division; Jack Mitchell, North Central Division; Joseph Fulton, Southern Division; John Heuberger, Potomac Division; and Thomas Sproston, Northeastern Division.

On February 15, a proposal was made to the National Science Foundation for the granting of funds to support preparation of the sourcebook. This project was approved by the National Science Foundation on April 9, 1959. A grant of $8,500 was made to the Teaching Committee to complete this project. The proposal included as the initial phase of the project a twelve-man Planning Conference at which the major phases of the sourcebook would be planned and a two-week writing conference for 6 persons to be scheduled about 1 year after the Planning Conference. The final draft of the sourcebook was to be completed within 18 months.

A proposal was made to the Teaching Committee to plan a Symposium on Teaching at the annual meeting at University Park, Pennsylvania. Approval for the Symposium was granted by the APS Program Committee, and a panel of 4 speakers and 4 discussion leaders was selected. The title of the Symposium is "Training for the Future in Plant Pathology."

Inasmuch as membership on the Teaching Committee did not include persons with commitments primarily in the field of forest pathology, a request was made and approved that 3 forest pathology teachers be added to the Committee.

In addition to the project on the preparation of a sourcebook, specific proposals are to be made at the annual meeting by each of the subcommittees of the Teaching Committee for other means of improving teaching in all areas of plant pathology. ARTHUR KELMAN, *Chairman*

Report of the Committee on Utilization of Plant Pathologists and Facilities in National Emergencies. The Committee reports the following activities and recommendations:

Activities—1) The Committee provided the President, APS, with an opinion relative to including the title of "Plant Pathologist" in the "List of Currently Critical Occupations" (as amended by Office of Defense Mobilization), U. S. Department of Labor. 2) It reported to the President, APS, that the title "Plant Pathologist" is entered in the "List of Critical Civilian Occupations for Screening the Ready Reserve," National Headquarters, Selective Service System, Bulletin 191. 3) It received no requests from plant pathologists in the armed forces for assistance in obtaining a scientific assignment.

Recommendations—1) It recommended that the APS channel through the American Institute of Biological Scientists any request that plant pathologists be included in the "List of Currently Critical Occupations." 2) It recommends that the Committee be continued for 1960.

H. H. THORNBERRY, *Chairman*

Report of the Committee on Virus Type Culture Collection. The second edition of the Catalog and Registry of the Plant Viruses was received for distribution in April, 1959. Of the 104 virus entries in the first edition, 29 were lost and not entered in the second edition. To the remaining 75 entries were added 77 new ones, making 152 entries in the second edition. In addition to the virus cultures, there are 59 entries for sources of seed and vegetative stocks of plants used in virus research.

The Committee's report, "Studies with commercially produced plant-virus antisera," is near completion and should be ready for submission to PHYTOPATHOLOGY for publication in the near future.

H. H. MCKINNEY, *Chairman*

Report of the Advisory Committee on Collecting and Disseminating Data on New Fungicide Tests. The Council approved the Committee's recommendations, and Dr. A. B. Groves, Department of Plant Pathology, Virginia Experiment Station, Winchester, Virginia, continued as coordinator of the project. As in the previous year, Dr. Groves had full responsibility in soliciting and preparing the data and manuscript for publication of the "Results of the 1958 Fungicide Tests." He also administered all other matters pertinent to the entire project, either directly or on consultation with, and advice of, the Committee.

Dr. Groves has continued with private publication of the "Report," as mentioned in last year's statement. This has enabled distribution and sales of the "Report" earlier than through previous methods of publication. This year, "Results of the 1958 Fungicide Tests" was ready in early March. Early publication has been of special benefit in making the "Report" more valuable in planning current-season experimental work.

The present "Report" has continued many of the features developed by Dr. Groves. Included are alphabetical indexing by state, province, or country, identity of individual reports, grouping reports under crop categories, and listing of materials tested and their source.

As in the past, publicity has been arranged about the "Report" and its availability. News releases appeared through the fine help and cooperation of *Plant Disease Reporter* and *Agricultural Chemicals*. Notices also appeared in *Croplife, Science, Journal of Economic Entomology, Journal of Agricultural and Food Chemistry, AIBS Bulletin, Better Farming Methods, American Potato Journal, American Fruit Grower, Crops* and *Soils, NAC News*, and PHYTOPATHOLOGY. Cards were also sent out directly by Dr. Groves to previous purchasers of the "Report." Such publicity has been felt to increase sales.

It is interesting to note that the "Report" has increased in size and coverage substantially since the 1956 edition. Involved with this has been a corresponding increase in cost, the greater part of which has been that of printing charges. A recapitulation follows:

Report for year	No. reports	No. pages	No. copies	Total cost	Cost per copy
1956	—	24	1500	$244	$.163
1957	66	44	1200	$375	.3125
1958	143	74	1300	$612	.471

Comments will be made later concerning these increases and their relation to the future program.

Dr. Groves' statement of the status of the project as of June 30, 1959, is as follows:

"I believe it of interest to note the substantial sales of the 1957 issue after the report was made last July. At this time 693 copies had been sold, and the sale of perhaps an additional 50 to 60 copies was anticipated. Actually, sales of the 1957 report now total 950. This made the project self-supporting even after the $200 honorarium was paid to me at the Committee's direction. Needless to say, this record was of considerable personal satisfaction.

"Sales of the 1958 report are ahead of that for the 1957 report of the same date last year. This is a reflection of substantially higher individual sales. Bulk-lot sales are about 165 behind last year. I believe some attention could profitably be given to this situation.

"A few copies of issues earlier than 1957 have been sold. Complimentary copies of the 1958 report were provided all cooperators. Requests for library exchange have been received, and I believe should be honored. This would be resolved automatically if the recommendations are adopted that the report be issued as a supplement to PHYTOPATHOLOGY.

"I have not attempted to allocate charges to each year's report, for sales of all issues are made simultaneously and expenses are to some extent incurred in the same manner. I therefore give a statement of sales receipts and expenditures since last July:

1958–59 income:		
Received from sale of 714 copies of 1958 reports		$689.20
Accounts due on sales of 1958 reports		33.45
	Total	$ 722.65
Received from sale of 257 copies of 1957 reports		256.64
Accounts due on sales of 1957 reports (Accounts due include those from previous year as yet unpaid)		11.35
	Total	$ 267.99
1958–59 expenditures:		
Printing of 1300 copies of 1958 report		$ 612.00
Printing of 400 card notices of 1958 report		5.00
Postage & parcel post charges		112.83
Stationery & mailing supplies		74.04
Stenographic & secretarial:		
Mrs. B. L. Ferguson		67.00
Miss S. A. Burgess		100.00
Honorarium to A. B. Groves		200.00
	Total	$1169.87

"Deposits for the past fiscal year totaled $907.11. Disbursements by check were $1067.49. Petty cash is $23.95. Balance of the account in the bank is $1190.20. Postage and a few minor items were paid for with currency received in orders. There are no outstanding bills. Correction has not been made for inventory on hand.

"These figures indicate the project to have 'gone into the red' by approximately $150.00 the past year. While this much more may be collected from past-due accounts and

additional sales, it is assumed that additional stenographic compensation will be paid.

"The added printing charge for the enlarged report will require substantially increased sales to keep the project self-supporting. Should industry choose to subsidize the project again at some future date, it might be accomplished through the purchase and distribution of a few hundred copies of the report rather than as an outright grant."

From the foregoing it is apparent that increased costs, and the anticipated continued trend in that direction, seriously affect the maintenance of this project on a self-sustaining basis. In addition, the amount of work entailed is reaching formidable proportions and more help may be required to lighten this load. The Council will recall that this project was originally underwritten by industry support in the amount of $2300. This sum is being gradually depleted in absorbing the losses incurred, with the exception of the 1957 "Report." No attempt had previously been made to compensate these losses by increasing the per-copy price for the "Report." We are now reaching a situation where certain considerations should be given to the entire future program of this project. With Dr. Groves' suggestions and discussion with the Committee, the following recommendations are being submitted for consideration, advice, or action by the Council:

1. Approval of continuance of the publication of results of fungicide tests.

2. Issuance of the "Report" as an annual supplement to PHYTOPATHOLOGY or other medium sponsored and controlled by the Society. The "Report" would be handled outside of the usual editorial channels and would continue to be available for sale as at present. This would provide an opportunity of bringing it to the attention of the entire mailing list of the Society. Such action would also give more offical publication status to the work, encourage submission of reports, improve the quality and extent of coverage, and provide for an area for collecting and presenting valuable information on fungicide activity. The Society would also benefit from the prestige of a publication valuable to both professional and industrial interests.

3. Increase the present price of $1.00 per copy to $1.50 or $2.00 per copy to cover mounting costs of printing and work attendant upon collecting and preparing the data and information for publication.

4. Commendation without qualification should be given Dr. Groves by the Council for the outstanding work he has done on this project.

5. Approval of the appointment of Dr. A. B. Groves to continue as coordinator for at least one more year. He has indicated his willingness to accept this assignment.

L. GORDON UTTER, *Chairman*

Report of the Advisory Committee on Integration of Research on Soil-Borne Pathogens. Because of the distances involved, this Committee was unable to meet prior to the writing of this report, but plans to do so at the International Botanical Congress in Montreal before the year's activities of the Committee are completed.

It is significant that each member of the Committee strongly recommends continuation of this Committee for another year in order to follow up the activities initiated in the past two years. These activities for the most part have been directed toward the integration of meetings on root diseases, regional representation of root disease investigators at these meetings, and the encouragement of such undertakings as the manual on "Methods for Studying Soil Microflora," published this year by Johnson, Curl, Bond, and Fribourg; and the annotated bibliography of root rot literature published in two parts (in 1958 and in 1959) by the Harrow Staff of the Research Branch of the Canada Department of Agriculture, of which Dr. L. W. Koch is director.

The Committee continues to feel that root disease research, because of its inherent difficulties, needs the encouragement and support of a central committee. Continuation of its present program calls for expanded efforts to arrange a symposium or colloquium on root diseases, to foster the interchange of personnel at meetings, and to collaborate with a subcommittee of the NRC that is concerned with the stimulation of research on the biological control of root diseases. Other questions the Committee has for consideration are whether to propose a prize for outstanding research in this area by a graduate student, to seek financial support for publication of bibliographies or current information, and how to meet the need for a center where identifications of soil fungi may be made and their biology studied.

The Committee recommends its continuance another year.

WILLIAM C. SNYDER, Chairman

Report of the Fungicide Advisory Committee. No specific requests have been received by the Committee during the year. The Secretary has referred several letters regarding source material to the Chairman, who answered without a need for consulting the Committee as a whole. The function of this Committee is to provide authorized opinions of the Society, particularly as regards labeling of fungicides and similar matters, as the need may arise.

S. E. A. MCCALLAN, Chairman

Report of the Special Committee for Services to Manufacturing and Editing Phytopathology. In October of 1959 the Committee, through its Chairman, called on the AIBS in Washington to discuss the possibilities that AIBS had under consideration for providing a "Central Services" program to its member societies. Individual societies were asked by AIBS to submit cost statements on their business and editorial offices as an aid in exploring the various possibilities. On December 8, 1958, the cost estimates of APS were submitted in letter to H. T. Cox. About March 1, 1959, AIBS obtained the services of F. C. Harwood, formerly of Waverly Press, who was to immediately contact editors, business managers, etc., of individual societies to discuss the various aspects of a "Central Service" program. No further report has been received from AIBS. The AIBS will probably move into new quarters in Washington, where such a program could be initiated with less overhead than could be possible in their present quarters.

G. S. POUND, Chairman

Report of the Advisory Committee on Agricultural Meteorology. The Committee was appointed in 1958 to serve in a liaison capacity between APS and the American Meteorological Society. The Committee has assumed the responsibility for notifying pathologists concerning recent developments in meteorology related to phytopathology and interesting meetings in meteorology.

One member of the Committee served as Chairman, another contributed a paper to the Second National Conference on Agricultural Meteorology, held at New Haven in 1958. Another member has been appointed to the World Meteorological Organization's working group on leaf wetness.

The Committee hopes to arrange symposia at national meetings of APS to acquaint the members with progress in meteorology of interest to pathologists.

P. E. WAGGONER, Chairman

Report of the Special Committee to Study the Possibility of Establishing a Center for the Maintenance, Distribution, and Study of Fungi and Other Microorganisms. This Committee has little activity to report, partly because of the magnitude of the assignment, and partly because of the Chairman.

The Committee has sounded out informally a number of individuals and a few organizations upon the idea of a microbiological center. In every instance the response has been strongly and enthusiastically in favor.

Two viewpoints on how to approach the realization of such a center have been offered. One is by obtaining aid from various foundations; the other is to seek federal funds. Along this line, it has been suggested that the

Society meanwhile back the Joint USDA-National Museum mycological program. Another suggestion is that we seek additional support for research in mycology already being conducted in various laboratories on a decentralized basis.

It seems desirable that the Committee look into the problem further before making any recommendations to the Society. WILLIAM C. SNYDER, Chairman

Report of the Resolutions Committee. WHEREAS, here in the shadows of Pennsylvania mountains, under the auspices of Pennsylvania State University, we have enjoyed a happy annual meeting, IT IS RESOLVED:

THAT The American Phytopathological Society expresses special appreciation:

a) To Dean Lyman E. Jackson for requesting the attendance of the AIBS; to former President Milton Eisenhower for making the facilities of the University available for this meeting; and to the present President, Eric A. Walker, for the continuing action that has made the meeting possible.

b) To Mr. Wayne R. Bechdel, who is responsible for the unusually effective registration facilities, including the station-wagon service whereby weak-backed members and their baggage were whisked to their abodes.

c) To the Local Arrangement Committee of the Dept. of Botany and Plant Pathology, who not only provided excellent entertainment through the courtesy of the Music Department but also held open house in their laboratories and placed instructive exhibits for the delight of the visitors.

THAT current Presidents of our Society who engage in selling future sites for future meetings be bonded, along with the Treasurer, as a protection against mishandled facts.

THAT high-up officials of our Society be relieved of the custom of wearing coats during general meetings where microclimates make the wearing of coats superfluous. These officials appear miserable enough without the coats.

THAT all future experiments on patent medicines for roses be conducted in the Pacific Northwest, where the hazards of growth will not militate against the harmony of the treatments, and that this recommendation be followed irrespective of the geographical location of the chairman of the Resolutions Committee.

FRANK P. MCWHORTER
GEORGE KENT
JOHN W. OSWALD

Report on Elections and General Business Meetings of APS and the Council. On Sunday, August 30, the Council reviewed the reports of APS committees, considered old and new business, and formed recommendations for consideration at the general business meeting. The ballots of the annual election were counted by a special committee appointed by President Glenn Pound and approved by the Council. The following were elected by majority vote: *President-Elect*, S. E. A. MCCALLAN; *Vice-President*, WM. B. HEWITT; and *Councilor-at-Large*, C. S. HOLTON.

By majority vote during the general business meeting, the Society approved the recommendations of the Council appointing the representatives, editors of PHYTOPATHOLOGY, and committees as listed earlier in this report under Organization of the American Phytopathological Society for 1960.

By majority vote the Society also approved the following recommendations of the Council:

1) That the Society again waive the $1,000.00 membership fee to the AIBS and pay $1.00 per member dues for 1960.

2) That the budget for the Committee on Public Relations be set at $1,500.00 for 1960.

3) That $100.00 be made available to the Committee on International Cooperation to help pay the costs of conducting Committee business.

4) That $300.00 be made available to the Committee on Phytopathological Reviews for completion of the 1960 (a 1-year extension of the 10-year) bibliography of review articles in plant pathology.

5) That the Treasurer of the Society be bonded at the expense of APS for a sum necessary to and commensurate with Society finances and business.

6) That the accounts and investments of APS and PHYTOPATHOLOGY be reviewed by a certified public accountant at least once in every three years.

7) That the Society prepare and publish a 10-year cumulative index for PHYTOPATHOLOGY for the years 1951–1960.

8) That the Fungicide Advisory Committee be discontinued.

9) That a Committee on Plant Disease Losses be formed, and that the following membership of the Committee be approved: RALPH SHAY, *Chairman*, J. CEDRIC CARTER, E. L. LECLERG, P. R. MILLER, A. L. SMITH, G. L. STOUT, and A. L. TAYLOR.

10) That an Ad Hoc Committee be formed to study the function and responsibilities of the various Society committees, and the organization and development of the programs of the annual meetings of the Society, and that the following membership of the Committee be approved: GLENN POUND, *Chairman*, W. C. SNYDER, S. E. A. MCCALLAN, RALPH SHAY, WM. B. HEWITT, GEORGE ZENTMYER.

It was further moved, seconded and passed that the Council prepare a statement of necessary alterations in the bylaws that will change nonmember subscription rates from $10.00 to $11.00 per year and submit the change to the membership for vote by ballot.

It was moved, seconded, and passed that the Council prepare a statement of necessary changes in the constitution and bylaws that will permit formation of a new division of the Society in the Caribbean area, and submit the change to the membership for vote by ballot.

Future meetings.—The Council confirmed the following meeting for 1960—52nd Annual Meeting, to be held at The American Baptist Assembly, Green Lake, Wisconsin, August 28-31, in conjunction with The Potato Association of America. An announcement concerning the 1961 and 1962 meetings will be made later.

THE OCCURRENCE OF A VARIETY OF ENZYMES HYDROLYZING CELL WALL POLYSACCHARIDES IN APPLES ROTTED BY BOTRYOSPHAERIA RIBIS

John H. McClendon, G. Fred Somers, and J. W. Heuberger

Accepted for publication September 28, 1959. Published as Miscellaneous Paper 338, with the approval of the Director of the Delaware Agricultural Experiment Station.

SUMMARY

Enzyme preparations from apples rotted by *Botryosphaeria ribis* (White Rot) were freed of non-colloidal material and freeze dried. These were compared with similar preparations from sound apples, tomatoes, and a commercial fungal "Pectinase" mixture. The rotten apple preparations hydrolyzed at varying rates the galacturonan, galactan, and araban moieties of pectin; they also hydrolyzed galactomannan (guar gum), xylan, and pectin ester groups, but apparently not cellulose. The sound apple or tomato preparations in some measure also hydrolyzed these substances, but the mode of action and change of activity with pH were frequently different. The sound apple preparations were in some cases (such as pectin esterase) at least as active as the rotten apple preparations. Evidence is cited for the existence in the preparations studied of several different enzymes all hydrolyzing galacturonan; the same appeared to be true with respect to galactomannan. More active preparations were usually obtained from buffer extracts of the press cake of the rotten apples than from the juice initially expressed.

Introduction.—To invade the interior of a soft plant tissue, a microbial pathogen must attack the middle lamella, and probably the primary wall of that tissue. In such an attack, it is customary to consider that enzymatic hydrolysis of the pectic substances is involved (21), presumably because they are well known and readily attacked by enzymes (6, 12, 13, 20). The middle lamella is also thought to be composed of an insoluble pectate, while the primary wall contains protopectin (4). Recent work has served to emphasize the variety of ways in which the galacturonan moiety (polygalacturonic acid) of pectic substances may be attacked by enzymes. Deuel and Stutz (6) have categorized these different enzymatic types as polygalacturonases I, II, and III. Type I is an endoglycosidase that rapidly liquefies pectate solutions. Type II is another endoglycosidase, but acts on fully methylated pectin. Type III is a saccharogenic exoglycosidase that hydrolyzes the terminal galacturonate groups of the pectate chain.

The enzymology of the galactan and araban moieties of pectic substances and of the hemicelluloses is, on the other hand, poorly known (18). It seems pertinent to point out that there is often as much or more hemicellulose as pectic substance in soft tissues (3).

In the experiments reported below, preparations from apples rotted by *Botryosphaeria ribis* Gross & Dug. were examined for their ability to attack various polysaccharides under various conditions and were compared with preparations from tomato fruit and sound apples and with a purely fungal "Pectinase" mixture. These experiments serve to emphasize: a) variety among the galacturonan hydrolases (polygalacturonases), b) the occurrence of enzymes that hydrolyze other polysaccharides, and c) the contribution of enzymes from the host, which presumably aid in the progress of the pathogen.

Materials and methods.—Rome Beauty apples naturally infected with *B. ribis* were permitted to become totally invaded. Inasmuch as the flesh remained a light cream color and the extracts from it did not discolor, it is presumed that this fungus destroyed most of the quinonoid precursors of the apples, which, when oxidized, are well known to be toxic to enzymes and to pectic enzymes in particular (5). The hard spot at the site of invasion was discarded, and the apples were pressed, finishing with a hydraulic press. The cake was extracted with 0.05*M* citrate buffer. The pH of the juice was about 3.5, and that of the cake extract was about 5.5. The clear juice and extract were ultrafiltered by osmotically forced dialysis against polyethylene glycol (14), dialyzed against distilled water, ultrafiltered again, and freeze-dried. The yield was about 0.4% (wt./vol.) of the juice and 0.1% of the cake extract (on the original juice basis). The dried colloidal material consisted mostly of polysaccharide. A sample of sound Rome Beauty apples was worked up in the same way (with similar yields) after freezing and thawing. Discoloration of the juice and cake extract was prevented here by the addition of ascorbic acid. A saline extract of whole, sound, ripe tomatoes was worked up in the same way. "Pectinase" purchased from the Nutritional Biochemicals Corporation was freed of dialyzable solids in the manner described above.

"Sodium Polypectate" was obtained from Sunkist Growers, Inc. (1). Pectin, pectic acid, and xylan were obtained from the Nutritional Biochemicals Corporation. Galactomannan was obtained as the ground endosperm of the guar seed ("Jaguar") from Stein-Hall, Inc., dispersed in water, centrifuged at 16,000 G, and precipitated with acetone. A pectate sample containing a high proportion of galactan and araban was prepared by standard methods from annual blue lupine seed (*Lupinus angustifolius* L.).

Paper chromatography of hydrolysis products on 5-in.-square sheets of Whatman No. 1 paper served as a test for the approximate extent of reaction. The solvent mixture used principally was n-butanol, pyridine, acetic acid, and water (6:4:1:3 v/v) (11, 16). Identification of the isomeric hexoses, pentoses, and hexuronic acids was performed on 18-in.-long sheets, using the above solvent, one in the proportions 7:7:1:6, or that recommended by Fischer and Dörfel (8). The color reagent was aniline trichloracetate in glacial acetic acid (9).

Enzymatic reduction of viscosity was followed by

TABLE 1.—Effect of pH[a] on enzymatic hydrolysis of pectate from blue lupine seeds by enzymes from different sources. Estimated from paper chromatograms.

Enzyme source[b]	pH 3	4	5	6	7
Galacturonic acid[c]					
Tomato	t	2	3	2	1
Apple	t	2	3	2	1
B. ribis + apple	3	3	3	3	2
"Pectinase"	5	5	5	5	2
Galactose[c]					
Tomato	t	6	7	6	1
Apple	t	t	1	1	t
B. ribis + apple	t	5	6	5	1
"Pectinase"	8	10	8	6	2
Arabinose[c]					
Tomato	t	2	3	2	
Apple	t	t	t	t	
B. ribis + apple	t	3	3	3	
"Pectinase"	8	10	8	4	2

[a] The composition of the mixture for hydrolysis was: 2% pectate, 0.05*M* buffer (citrate at pH 3, 4, 5, 6, phosphate at pH 7, tris(hydroxymethyl)aminomethane at pH 8, 9), 20 mg. ml. enzyme (2 mg./ml. of "Pectinase"). Room temperature (about 26°C) was used. pH 8 and 9 showed no products, and are therefore omitted.

[b] For the preparations from the juice of sound and rotten apples, the reactions were similar to but slower than those for the cake preparations shown here.

[c] The numbers in the table are approximate estimates of the relative amount (from 1 to 10) of product present after 24 hours, except for "Pectinase," with which 3–6 hours were sufficient. "t" indicates minimum quantities possibly not due to enzymatic hydrolysis.

measuring the outflow time of a capillary pipette viscometer (2). The reciprocal of the elapsed time to reach a given outflow time was proportional to enzyme concentration.

Results.—*Pectic glycosidases.*—Table 1 shows the results of paper chromatography of the enzymatic hydrolysis products of the lupine seed pectate at various pH values. The amounts of the split products were estimated only roughly, by eye. It is evident, first of all, that the strictly higher plant preparations (especially from tomato) produced appreciable amounts of galacturonic acid, galactose, and arabinose. Indeed, the sound and rotten apple preparations produced galacturonic acid at rather similar rates at pH 5, but at markedly different rates at pH 3. On the other hand, the sound apple preparation produced much less galactose than did the rotten apple and tomato preparations. Only the "Pectinase" hydrolyzed the araban rapidly.

Qualitatively, the various preparations showed a greater contrast in behavior at pH 3 than at pH 4 or 5. Thus, sound apple and tomato preparations produced very little galacturonic acid at pH 3, even after long incubation, whereas rotten apple preparations (and "Pectinase") produced galacturonic acid as fast at pH 3 as at higher pH's. In the case of galactose and arabinose, only the "Pectinase" was highly active at pH 3. Another prominent difference distinguishing the fungal preparations was the occurrence in these alone of an apparently acidic compound giving the color of pentose (red) but moving more slowly than galacturonic acid on the chromatogram. This was most prominent at pH 4 and 5. The unknown was not the same as that reported to accumulate in overripe apples (16).

Fig. 1 shows activity vs. pH curves for enzymatic viscosity reduction of "polypectate." The lower leg of the curve descends unreasonably steeply and may be an artifact. (The viscosity of the "polypectate" itself, while rather constant down to pH 4.3, rose steeply below that point.) The upper leg of the curve, on the other hand, very clearly shows a marked difference between the properties of the rotten apple cake and juice preparations. The latter has a single activity maximum (pH 4.3). Subtracting this from the curve for the cake preparation reveals a maximum at pH 5 and a shoulder at pH 6.

In another experiment, viscosity reduction and reducing-group production (10) were followed concurrently, using "polypectate" at pH 4, and a few samples were taken for paper chromatography. The rate of reduction of viscosity relative to the initial rate of reducing-group production was about twice as great in a rotten apple cake preparation as in a rotten apple juice preparation. In both preparations the occurrence of oligosaccharide intermediates (6) was observed on the paper chromatograms.

Paper electrophoresis (at pH 6) of some of the

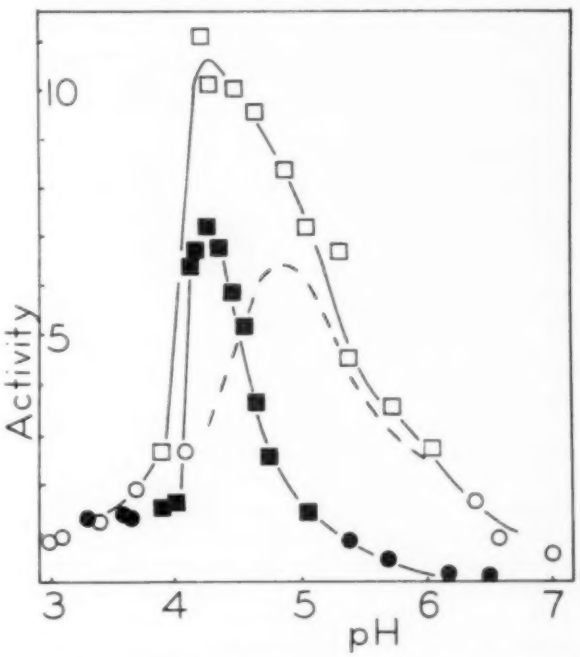

Fig. 1. Effect of pH upon rate of viscosity reduction of high-viscosity pectate ("polypectate") by enzymes from *B.-ribis*-rotted apples. Filled symbols denote a juice preparation, and open symbols a cake preparation. Dashed line represents the difference between the two experimental curves. The reaction was followed at 25°C in test tubes containing 5 ml of 0.5% sodium "polypectate" in the appropriate buffer (.05*M* citrate), plus 2 ml of the enzyme solution. The end point taken was 75% reduction of the initial viscosity, with activity expressed as $(\text{min.})^{-1}$ (mg. enzyme)$^{-1}$ × 10. Squares show averages of 3 or 4 replicates; circles show individual measurements.

rotten apple preparations showed that the pectate glycosidase activity prominent near pH 4 moved rapidly toward the anode, whereas that prominent near pH 6 moved only slowly in the same direction. The enzymes were detected by placing the paper strips on buffered agar plates containing pectate, the unaffected portion of which was precipitated with acid (19). The fast-moving enzyme did not attack pectin (17). The slow-moving one did because of the presence of either pectin esterase or a Type II enzyme.

Viscosity reduction of "polypectate" by the sound apple preparations was detectable, but was apparently less than 1% as fast as the rotten apple preparations at either pH 4 or 6. This is in marked contrast to the rate of galacturonic acid production, which was of the same order of magnitude in both sound and rotten preparations, except at very low pH.

Pectin esterases.—Since the pectin esterase of higher plants differs markedly from that of fungi (12) in its pH behavior, studies were made at pH 4.5 and 6 (in the presence of 0.1*N* NaCl to activate the enzyme from the higher plant). The activities of the rotten apple preparations and the tomato preparations were all 4–5 times as great at pH 6 as at pH 4.5. A trial of a sound apple cake preparation at pH 6 showed it to be more active than the rotten apple preparations. One cannot now say whether there is no fungal pectin esterase produced in these rotting apples, but the low activity at pH 4.5 shows that there was a preponderance of the esterase from the apple itself. The rates at pH 6 of two cake preparations were 0.01 and 0.03 meq./min./g enzyme.

Galactomannan glycosidases.—Viscometric assay of galactomannan hydrolysis at several pH's showed activity maxima near pH's 4 and 6, with the ratio of the two activities (4/6) apparently varying between 1.5 and 9 for different preparations. Although activity was found with both sound apple and tomato preparations, the rotten apple preparations were more active. Furthermore, the above-mentioned ratio was smaller with the preparations from the fungal sources (including Pectinase).

The viscosity-reducing activities of several enzyme preparations at pH 4.3 fell in the following sequence (for an equal weight of enzyme): rotten apple cake > sound apple cake > rotten apple juice > tomato = sound apple juice. However, when hydrolysates at pH 4.3 were examined for free hexoses, the order was tomato > sound apple cake > rotten apple cake (trace). The rotten apple cake produced no more hexose at pH 6. The predominant hexose in the tomato hydrolysate was galactose, with a small amount of mannose, but less than 5% of the galactomannan appeared as free hexose. The galactose all occurs in single-unit side chains (20), so it is likely that viscosity reduction and galactose production are unrelated.

Other polysaccharide hydrolases.—Cellulose dialysis tubing was used routinely during the preparation of the enzymes, and at no time was there any suggestion of weakening from contact with the rotten or sound apple preparations, although there was with Pectinase.

Using paper chromatography, xylan hydrolysis was undetected with rotten apple juice preparation, was slow with a rotten apple cake preparation, and was much faster with Pectinase. Some glucose polymer accompanying the xylan was also hydrolyzed by all three in similar ratio. The rate of hydrolysis of the xylan was greatest between pH 3 and 5. The sound fruit preparations were not tested against xylan, but traces of xylose appeared in the lupine seed pectate digests, reported above, especially with the tomato enzymes at pH's 4, 5, and 6.

At pH 3.5, fat-free ground endosperm of the coconut (20) was hydrolyzed by "Pectinase" to mannose and less glucose, galactose, and galacturonic acid. However, rotten apple juice and cake preparations yielded only the same small amount of galacturonic acid and traces of hexoses.

Autolysis.—The rotten apple preparations consisted mostly of polysaccharide that was only slowly hydrolyzed by the enzymes it contained. To determine the composition of this resistant polysaccharide, a sample of a rotten apple juice preparation was hydrolyzed by acid and by Pectinase. The acid hydrolysate contained a uronic acid, galactose, arabinose, xylose, and rhamnose, in decreasing amounts. A carbazole test for uronic acid (7) indicated some 40% uronic acid. The enzymatic hydrolysis (pH 3.5) yielded arabinose and galacturonic acid plus an unidentified compound similar to that produced from the lupine seed pectate. Some of the contaminating polysaccharide probably could be eliminated by incubation at the proper pH. However, some ultrafiltered Pectinase preparations contained as much as 75% polysaccharide.

Tissue maceration.—As noted elsewhere (15) tissue maceration by these fungal enzymes has a pH optimum near 3.3. On a dry-weight basis, the rotten apple cake preparations were about 10 times as active as the rotten apple juice preparations. No activity was detected (at pH 3.5 or 4.5) in the corresponding sound apple preparations, but the tomato enzymes macerated at pH 4.5–5.0.

Discussion.—The most obvious source of heterogeneity in the samples of enzymes derived from the rotten apples lies in the dual nature of these preparations. Some of the enzymes apparently came from the fungus, and others from the apple fruit. The quantitative significance of the latter source has not been assessed precisely. It is possible that more apple enzymes were extracted from the rotten apples than from the sound apples; that is, some enzymes might be trapped in the unhydrolyzed matrix of cell wall material, but released when the latter is attacked by fungal enzymes. The possible role of the fungus in destroying toxic compounds has already been mentioned, and may have been a factor in the poor recovery of certain enzymes from the sound apples.

It is apparent from the pH-activity curves (Fig. 1) that the glycosidases attacking pectate solutions are diverse in nature. On the basis of the evidence at hand, these are probably all Type I polygalacturonases.

Type II, which might be present, could not be tested for in the presence of the pectin esterase. Type III (the exoglycosidic polygalacturonases) apparently also occurred, especially in the sound-apple preparations where viscosity reduction was almost undetectable. The varying ratio of viscosity reduction to reducing group production in the rotten-apple preparations also indicates the presence of Type III enzyme.

The acidic hydrolysis product (from lupine pectate), which gave a color reaction like that from pentose, was possibly derived from a branch point between the galacturonan and araban chains. If so, it is remarkable that, though this substance was produced in good yield by the rotten-apple-cake preparation, little if any was liberated by the sound-fruit preparations. The hydrolysis of araban itself did not show this distinction. —Department of Agricultural Biochemistry and Food Technology, and Department of Plant Pathology, University of Delaware, Newark.

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ADAPTATION OF THE CORN LEAF BLIGHT FUNGUS TO A RESISTANT AND A SUSCEPTIBLE CORN HOST

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SUMMARY

Twenty-five single-conidial isolates of *Trichometasphaeria turcica*, each from a susceptible inbred line of corn, K64, and its resistant counterpart, CI.64, were tested separately on these 2 inbred lines of corn. The range of mean infection scores was 1.81–2.08 for isolates from K64 and 1.79–2.07 for isolates from CI.64. When separate analyses were calculated for the 2 hosts, virulence of the isolates from the 2 sources on CI.64 hosts was significantly different. Differences in virulence of the isolates from the 2 sources on K64 were not measurably significant. Isolates from the 2 sources, on the average, differed in their virulence on the 2 hosts. Some of the isolates within a source failed to follow the average pattern.

The northern corn leaf blight fungus, *Trichometasphaeria turcica* Luttrell (*Helminthosporium turcicum* Pass.), has shown evidence of physiologic specialization on different strains of corn (7). An earlier study (6) showed that there were variations in the virulence of single-conidial and hyphal-tip isolates of the fungus on one strain of corn. Whether they were due to genetic differences in the fungus was not established. In other studies Jenkins and co-workers (1, 2, 3) reported genetic differences in the susceptibility and resistance of inbred lines of corn. Resistance can be transferred to a susceptible line by breeding (4). Major differences between the virulence of the same group of isolates on two separate hosts could be due to genetic differences of the hosts in susceptibility and resistance to the fungus. Differences of virulence of two groups of isolates on the same host are a result of variation, some of which may be caused by variation

Table 1.—Results* of inoculating the susceptible K64 and the resistant CI.64 corn inbred lines with conidial isolates of *Trichometasphaeria turcica* obtained from each line

Isolates from	Means			Isolates from	Means		
K64	CI.64	K64	Both	CI.64	CI.64	K64	Both
4	0.07	3.55	1.81	28	0.07	3.51	1.79
19	0.02	3.64	1.83	44	0.10	3.54	1.82
3	0.04	3.64	1.84	47	0.05	3.60	1.83
25	0.04	3.67	1.85	31	0.08	3.60	1.84
2	0.06	3.63	1.85	43	0.07	3.61	1.84
18	0.12	3.61	1.87	36	0.12	3.58	1.85
9	0.10	3.68	1.89	26	0.14	3.59	1.87
24	0.08	3.71	1.89	46	0.08	3.66	1.87
20	0.08	3.76	1.92	49	0.08	3.66	1.87
8	0.05	3.82	1.94	32	0.09	3.67	1.88
10	0.15	3.75	1.95	38	0.10	3.68	1.89
14	0.09	3.81	1.95	40	0.14	3.64	1.89
12	0.08	3.84	1.96	27	0.20	3.60	1.90
11	0.07	3.88	1.98	42	0.11	3.72	1.91
23	0.15	3.80	1.98	48	0.12	3.69	1.91
5	0.09	3.89	1.99	45	0.13	3.71	1.92
6	0.04	3.94	1.99	29	0.09	3.77	1.93
22	0.11	3.87	1.99	35	0.12	3.75	1.93
16	0.08	3.92	2.00	50	0.08	3.77	1.93
17	0.12	3.93	2.03	33	0.07	3.86	1.96
21	0.08	3.97	2.03	34	0.09	3.92	2.00
7	0.11	4.06	2.08	41	0.10	3.95	2.02
				37	0.12	3.95	2.04
				30	0.13	4.00	2.06
				39	0.09	4.05	2.07
Means	0.08	3.79	1.94		0.10	3.72	1.91

* In the scoring, 0 represents no infection and 5.0 represents the most infection.

in the genetic makeup of the fungus isolates related to specificity to the hosts. This study was designed to determine if single-conidial isolates of *T. turcica* from susceptible line K64 and resistant line CI.64 exhibited specificity in their infection of these 2 hosts.

Materials and methods.—*Source of inbred lines.*—Seed of the two inbred lines used was produced at the Plant Industry Station, Beltsville, Maryland. Inbred line K64 was developed at Kansas Agricultural Experiment Station from a white variety, Pride of Saline. K64 is susceptible to northern leaf blight of corn. Inbred line CI.64, which is resistant to northern corn leaf blight, originated at the Plant Industry Station. It was produced by crossing K64 with a leaf blight resistant line, Mo21A, and backcrossing to K64 three times.

Source of isolates.—The *T. turcica* isolates came from the inbred lines K64 and CI.64, which were artificially inoculated in the field with *T. turcica* in 1957. This artificial epidemic was produced by using a composite inoculum of 22 isolates from 14 states of the United States. K64 became heavily infected, and CI.64 had none to a few lesions per plant. Leaves with blight lesions from these 2 inbred lines were collected in the fall of 1957 and stored at about 12°C until June, 1958. Single-conidial isolates were then made from spores produced in moist chambers from 25 separate lesions each from K64 and CI.64. One isolate from each of the 50 lesions was used in the experiment.

Inoculations.—Cultures were grown for 3 weeks in 90-mm Petri dishes on potato-dextrose agar. Concentrated inocula were prepared by grinding each culture, including the agar medium, for 2 minutes in 200 ml of distilled water in a Waring Blendor, and diluting to make 500 ml. For inoculation in the field these concentrates were diluted 8 times.

Inoculations were made by pouring about 10 ml of inoculum into the leaf whorls when the plants were 7 weeks old. Two inoculations were made with each isolate, 4 days apart. Cultures for the second inoculation were made from the original cultures after 5 days' growth.

Isolates were tested individually on both lines of corn. The experiment was laid out in a split-plot design with 4 replications. There were 50 whole plots corresponding to the 50 isolates. Each whole plot contained 2 sub-plots, each consisting of a single row, 25 hills long, with one plant per hill. These 2 sub-plots consisted of one row of the inbred line K64 and one row of CI.64. Whole plots within each replication and sub-plots within each whole plot were randomized.

Data were taken on the severity of initial leaf infection 18–20 days after inoculation. The first 20 plants of each sub-plot were scored individually for initial leaf blight infection in 11 classes of blight intensity. The scoring was similar to that used in late-season ratings (6) except that the classes were modified slightly. No infection was represented by 0, and the ten other ascending scores up to 5.0, in steps of 0.5, represented increasing amounts of leaf blight.

Results.—The infection of the isolates on each host is listed in the order from low to high mean infection ratings on the 2 hosts (Table 1). Data from isolates 1, 13, and 15 from K64 are eliminated because of probable errors in inoculation procedures. Mean infection scores on the 2 hosts ranged from 1.81 to 2.08, averaging 1.95, for isolates from K64, and from 1.79 to 2.07, averaging 1.91, for isolates from CI.64.

The scores of individual plant ratings of CI.64 produced a highly skewed distribution. The distribution of plot means, however, appeared essentially normal. All statistical analyses were done on plot means. Since the mean blight scores of the 2 hosts were dissimilar, it was felt that heterogeneity of variances would invalidate the usual tests of significance. To avoid this difficulty, separate analyses were calculated for the K64 host and the CI.64 host. On the CI.64 host the differences between sources of inoculum were significant at the 5% level and closely approached significance at the 1% level. On the K64 host the differences between sources of inoculum were not significant.

Possible interactions involving lines, sources, and isolates were of particular interest, but these could not be estimated and evaluated from a routine combined analysis, because of the heterogeneity of variances. The analysis used involved the differences between K64 and CI.64 to satisfy the postulates for analysis of variance.

The lines × sources interaction was significant at the 5% level. This indicated that the 2 sources,

averaged over all isolates, differed in their average on the 2 hosts. This is demonstrated by a tendency for the isolates from a particular host to be more pathogenic on that host. The interaction, lines × isolates within sources, was highly significant. This indicated that some of the isolates within a source failed to follow the average pattern.

Discussion.—The resistance and susceptibility of corn to *T. turcica* is determined by genetic differences in the hosts (4, 5, 7). The inbred line, K64, is susceptible to the fungus, and CI.64 is its resistant counterpart. They are assumed to be genetically similar except for their reaction to northern corn leaf blight. Previous reports (6, 7) on work with the fungus show variability in pathogenicity on corn inbred lines. It has been indicated that a significant amount of heterogeneity was genotypic when conidial isolates were used. However, variability was not sufficiently stable to be used as a criterion for establishing specific races. The results herein reported show that the differences in mean leaf-blight ratings of all the isolates on each of the 2 inbred lines were small. These differences were measurably significant, however, as indicated by the interaction of lines × sources. Isolates from K64 were slightly more virulent on K64 and less virulent on CI.64 than were isolates from CI.64. As would be expected with such a variable fungus, not all isolates conformed to the average pattern of reaction.

Not much is known of the effect of the host on the virulence of fungi. No data are available for *T. turcica*, except as reported in a study of its variability (6). Passage through a susceptible host increased virulence in some instances and decreased virulence in others. This characteristic of the fungus was not stable. Sussman (8), in reference to the adaptation of fungi in general, claimed that genotypic adaptations are generally made over long periods. Phenotypic adaptations may be made within one or several generations. Some type of adaptation has been measured in the present study. Whether these adaptations would progress through additional generations of the fungus in the same direction could only be determined by further testing. From this and previous studies there is little doubt that some of the variability, specificity, and adaptability of *T. turcica* for different hosts is due to genetic influences of the fungus.—Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

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CORYNESPORA BLIGHT OF SESAME

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SUMMARY

A *Corynespora* species pathogenic on sesame in Mississippi appeared identical to the one causing target spot of soybean and agrees with the description of *C. cassiicola*. Cross-inoculations succeeded with isolates from sesame and soybean. The morphology of the conidia was altered on the different hosts, being consistently wider on sesame than on soybean. The fungus overwintered on sesame and soybean plant debris in the field, and was carried on and within the seeds.

Premature defoliation and death of sesame, *Sesamum indicum* L., occurred at Stoneville, Mississippi, in the fall of 1958. The leaves of infected plants had large, irregular-shaped, light-brown lesions that later coalesced. As leaf infection progressed, the plants were defoliated. Infection of the stems caused light-brown elongated lesions that spread over the entire plants. By October, the sesame was killed. Consistently obtained from the leaves and stems of infected plants was a fungus that corresponds with Wei's (7) description of *Corynespora cassiicola* (Berk. & Curt.) Wei. Previous to Wei's description of the genus *Corynespora* the fungus had been described as *Cercospora vignicola* Kawamura (2), as *Helminthosporium vignae* Olive, Bain & Lefebvre (6), and as *Helminthosporium vignicola* (Kawamura) Olive (5). Other epithets that were placed in synonomy by Wei are *Helminthosporium cassiicola* (Berk. & Curt.) Berk., *Helminthosporium papayae* H. Sydow, *Cercospora melonis* Cooke, and *Corynespora mazei* Gussow.

Corynespora has not been reported previously on sesame in the United States, nor has the fungus been

proved to be pathogenic on sesame in the field elsewhere. Mohanty and Mohanty (4) isolated the fungus from sesame leaf spots in India, but did not test its pathogenicity on sesame. Wei (7), at the Commonwealth Mycological Institute, studied herbarium material that had been collected from sesame leaves in Morogoro, Tanganyika Territory.

The fungus was first reported in the United States in 1945, on cowpea, *Vigna sinensis* (Torner) Savi, and soybean, *Glycine max* (L.) Merr. (6). The common name of "target spot" was given the disease, because of the concentrically zoned leaf spots incited by the pathogen. Cross-inoculation studies with isolates from these hosts on cowpea and soybean indicated possible race differences. The isolates from cowpea were reportedly more virulent on the two hosts than were those from soybean. Consequently Races 1 and 2 were designated from cowpea and soybean, respectively. Since that report by Olive et al (6), target spot has been prevalent and destructive on soybeans in the alluvial soil area adjacent to the Mississippi river in Louisiana, Mississippi, and Arkansas (1).

During the 1958 season at Stoneville the fields of sesame infected by *Corynespora* were adjacent to fields of target-spot-infected soybeans. Because of the proximity of infected soybeans and the similarity of the two fungi, an investigation was undertaken of the pathogenicity of the sesame fungus and its possible identity with the soybean organism.

Material and methods.—The sesame isolates used were single-conidium isolates made in October, 1958, from stems of plants naturally infected in the field. Soybean isolates were likewise single-conidium isolates also made in October, 1958, from soybean leaflets infected in a natural epiphytotic of target spot at Stoneville. The isolates were maintained on tubed PDA (potato-dextrose agar). Transfers were made as infrequently as possible since the organism has been reported (6) to lose pathogenicity on repeated transfer.

Since *C. cassiicola* is reported to have a wide host range, 4 plant species were inoculated to compare the isolates from sesame and soybean. Plants used were sesame (Margo), soybean (Experimental line N54-1748), okra, *Hibiscus esculentus* L. (Perkin's Mammoth Long Pod), and cowpea (Early blackeye). In each inoculation series 8–12 plants of each species were inoculated with the respective isolates; 4–6 plants of each crop were used as controls. Plants were 30–60 days old when inoculated.

Inoculum was prepared by macerating Petri-dish cultures of the fungus in a food blender at a ratio of 1 dish per 200 ml distilled water plus 1 drop of detergent (Triton X-100). The plants were sprayed with inoculum until uniformly wet. Control plants were sprayed with a solution of sterile PDA in distilled water and detergent at the same concentration as the inoculum. The sprayed plants were placed in a moist chamber at 100% relative humidity for 24 or 48 hours and then removed to a greenhouse bench and observed for symptoms. Three cross-inoculation series were conducted. Cultures used in the first 2 were isolates obtained from plants infected in the field. Re-isolations of the sesame and soybean isolates were made from sesame leaves infected by the respective isolates in the second inoculation series. These re-isolates were then used as inoculum in the third inoculation series.

Conidia for measurement studies were obtained from greenhouse-inoculated sesame and soybean leaves infected by the sesame and soybean isolates. These leaves were placed in a moist chamber and periodically observed with a steroscopic microscope until conidia were abundant. Conidia-bearing sections were then mounted in lactophenol on microscope slides. The width and length of 50 conidia of each isolate on each host were measured. The width was measured at the widest part of the conidia; the bulbous base just above the hilum. The length was taken from tip to tip. Conidia for germination studies were mounted on deep well slides in hanging drops of sterile distilled water. These conidia were obtained from leaf lesions produced by inoculating the sesame and soybean isolates on sesame and soybean leaves.

Experimental results.—*Pathogenicity studies.*—In the first 2 inoculation series, lesions were apparent on all inoculated plants within 3–4 days of inoculation, whereas control plants were disease free. The sesame and soybean isolates were identical in pathogenicity on a given host; host reaction, however, differed. Sesame was the most severely infected, with the leaves appearing scorched or with large, coalescing, light-brown lesions. These lesions developed a purple or reddish-brown margin, and the centers blanched with age (Fig. 1-A). Many infected leaves turned yellow and abscised. Stem lesions were long, reddish-brown streaks with light-brown centers that often coalesced, blighting the plants (Fig. 1-B). Both leaves and stems of soybean and okra were infected, whereas only the leaves of cowpea were infected. The lesions on soybean and okra appeared similar to those on sesame, but were not as large or extensive (Fig. 1-C,D). Lesions on cowpea leaves were usually small (1–1.5 mm diameter) and were dark red on the underside of the leaf but did not often develop the light-brown center (Fig. 1-E).

The results obtained in the third inoculation series with the reisolated cultures were comparable to those obtained in the first 2 inoculations. Pathogenicity of the 2 isolates was again equal on the same host.

Conidial measurements and germination.—Measurements of the 4 groups of conidia showed conidial lengths to be quite variable throughout, with no difference between groups. The range in length was 39.0–280.8 μ, with the usual range 120.9–218.4 μ (145 of 200 conidia measured) and a mean of 153.8 μ. The width of the conidia, however, differed significantly between the 2 hosts, but those of the 2 isolates did not differ when from the same host (Table 1). The sesame and soybean isolates produced wider conidia on sesame than on soybean. Table 1 presents an analysis of variance based on single degrees of freedom for these comparisons. Apparently the host affects the width of the conidia produced by the organism.

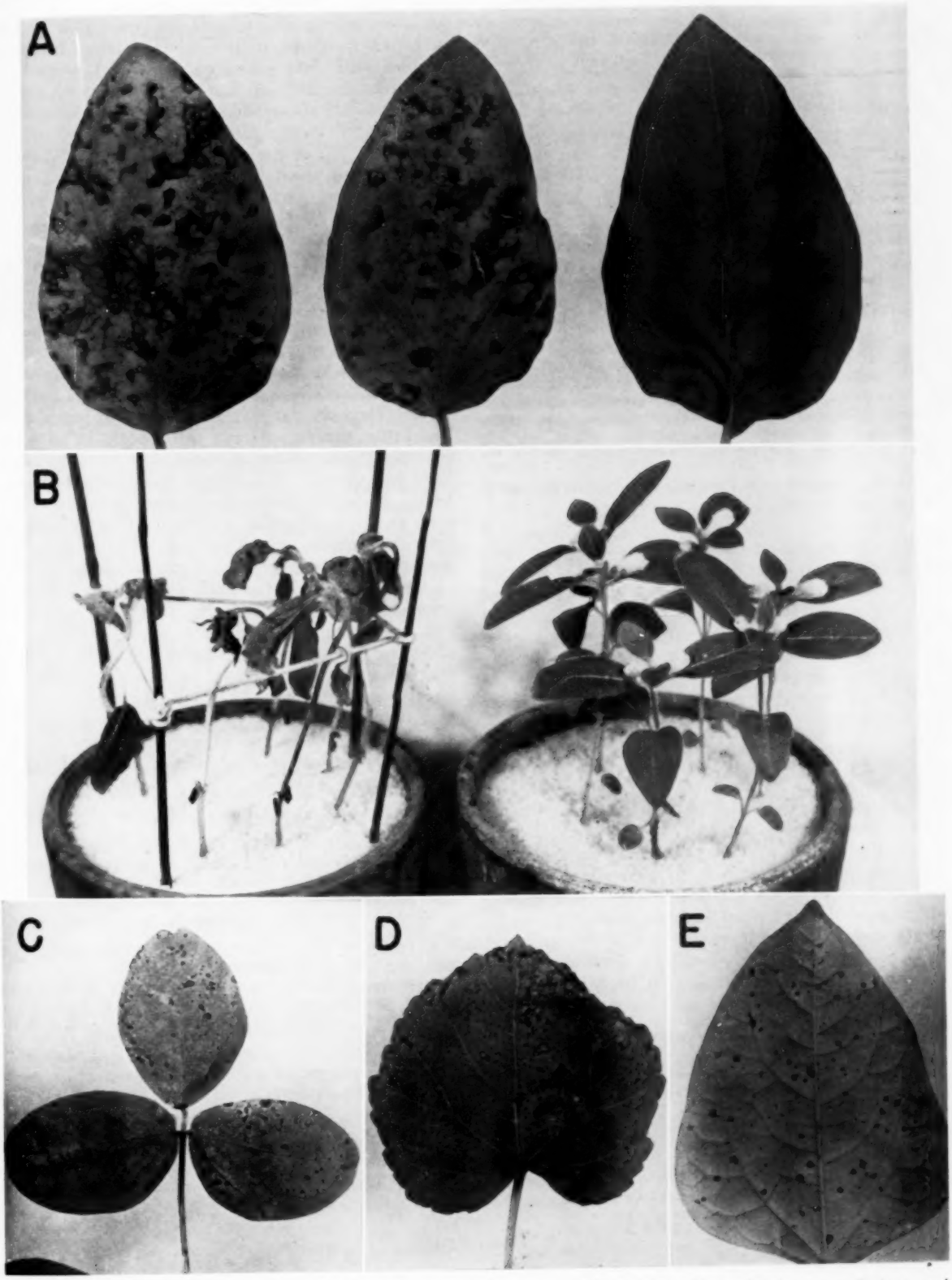

Fig. 1. Symptoms produced on host plants inoculated with the *Corynespora* isolated from sesame. **A)** Sesame leaves illustrating typical leaf spotting. Note coalescing lesions and those with light-colored centers. Leaf on right is control. **B)** Sesame plants showing stem lesions and blighting of plants. Plants on right are controls. **C)** Inoculated soybean leaf. **D)** Inoculated okra leaf. **E)** Inoculated cowpea leaf.

Table 1. Widths of conidia of Corynespora cassiicola isolates from sesame and soybean leaves, and analysis of variance of the measurements

Isolate and host[a]	Mean width[b] (μ)	Range (μ)
A	10.06**	5.8–12.8
B	8.26	6.2–11.2
C	10.02**	6.4–12.8
D	8.93	6.4–11.2

Source of variation	Analysis of variance (degrees of freedom)	Mean square[c]
Groups	3	38.6**
A+B vs C+D	1	4.9
A+C vs B+D	1	104.5**
A+D vs B+C	1	6.4
Individuals	196	2.0
Total	199	

[a] A, sesame inoculated with sesame isolate; B, soybean inoculated with sesame isolate; C, sesame inoculated with soybean isolate; D, soybean inoculated with soybean isolate.
[b] Mean of 50 conidial measurements; double asterisks mark the means that are significantly higher at the 1% level.
[c] Double asterisks mark the mean squares that are significant at the 1% level.

Germination studies showed that some conidia could germinate within 2 hours of being mounted in hanging drops of distilled water. At 24 hours, the majority had germinated from the polar cells, with germ tubes about the length of the conidia. After 48 hours several conidia were found to produce germ tubes laterally from the middle cells, as previously reported by Olive et al (6). There were no apparent differences among the 4 groups of conidia in rate and type of germination.

Overwintering and seed transmission.—Sesame stems were collected in the spring of 1959 from a fallowed field on which infected sesame had been grown the year before. These stems were taken into the laboratory and placed in a moist chamber. Two days later they were examined with a stereoscopic microscope and were found to be producing *Corynespora* conidia abundantly. Overwintered soybean stems infected by target spot, collected from the field in the spring of 1956, produced *Corynespora* conidia when placed in a moist chamber for 24–48 hours. It is obvious that *C. cassiicola* can overwinter in host plant debris on the soil surface.

Liu (3) reported isolating the fungus from discolored soybean seeds collected in China in 1945–47. Isolations were made at Stoneville in 1958 from discolored soybean seed produced by plants heavily infected by target spot. The seed were surface-sterilized for 30 seconds in 50% ethanol, followed by 60 seconds in 1/1000 mercuric chloride, given 4 rinses in sterile distilled water, and plated on PDA. *Corynespora* was obtained from 17% of the 156 soybean seed plated. Isolations were also made from randomly selected seed from infected sesame plants. When surface-sterilized, fewer than 1% of the sesame seed produced *Corynespora*, though about 5% produced the fungus when plated without surface sterilization. These results indicate that *C. cassiicola* is carried both on and within sesame and soybean seed.

Discussion.—The experimental data show that the *Corynespora* sp. that attacks sesame is identical to *C. cassiicola*, which causes target spot of soybean. Olive et al (6) distinguished 2 races of the fungus. Race 1 caused severe infection of cowpeas and light spotting on soybean, whereas Race 2 caused few to many very small spots on cowpeas and light spotting of soybean leaves. The only sign of stem infection in any of their inoculation experiments was the appearance of a few superficial spots on the stems of some cowpeas inoculated with Race 1. The isolates from sesame and soybean used in this study caused severe infection of soybean and light infection of cowpea. They also caused extensive stem lesions on soybean. Johnson et al (1) reported that the target spot organism caused spotting of soybean stems in the field, and stem lesions have been observed by the authors. There is a possibility that the isolates studied may be of another race. However, since comparisons were not made with the organisms originally isolated by Olive et al, there is no assurance that an additional race exists. Race 2 and the sesame and soybean isolates may give the same results from controlled comparative inoculations. —Delta Branch of the Mississippi Agricultural Experiment Station, Stoneville.

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ISOLATION OF A FUNGITOXIC COMPOUND FROM CARROT ROOT TISSUE INOCULATED WITH CERATOCYSTIS FIMBRIATA

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SUMMARY

A crystalline compound toxic to *Ceratocystis fimbriata*, a fungus non-pathogenic to carrots, has been isolated from carrot root tissue previously inoculated with this fungus. The presence of this compound in inoculated tissue appears largely responsible for the resistance of the carrot to attack by *C. fimbriata*. This compound has been partially characterized with respect to its physical and chemical properties.

Introduction.—In several instances, the resistance of plants to fungus diseases has been reported to be due to the presence of chemical substances in the plants. Two different mechanisms appear to be responsible for this resistance. In one mechanism, the compounds are present in the prospective plant host before attack, and prove toxic to the fungus prior to its penetration of living host tissue. Angell, Walker and Link (1), and Link and Walker (4) showed that protocatechuic acid and catechol in the outer dead scales of colored onions made them resistant to onion smudge and neck rot caused by *Colletotrichum circinans* (Berk.) Vogl. and *Botrytis allii* Munn. These compounds were not present in white onion scales, and the white onions were susceptible to the diseases. In the other mechanism, plants produce toxic compounds only when the attacking organism has penetrated the host's cells, and these compounds appear to be produced only at the sites of infection. The disease resistance in this case seems to involve the interaction of the living plant host and the attacking organism.

It has recently been reported that white potato tissue resists the attack of several fungi, normally nonpathogenic to the potato, by producing fungitoxic phenolic compounds such as chlorogenic acid and caffeic acid. These were not present in the tissue before inoculation with the fungi, and appear to be localized in the areas of fungus penetration (3).

This paper reports progress on a biochemical study of disease resistance mechanisms operative in plant tissue. A defense mechanism is suggested for the resistance of carrot root tissue to attack by *Ceratocystis fimbriata* Ell. & Halst., a fungus nonpathogenic to carrots but an incitant of black rot in sweet potatoes.

Experimental.—*Preparation of carrot root tissues.*—Commercial varieties of carrots (predominantly Imperator and Long Imperator), free of visible surface defects, were washed with detergent and water. Peel tissue (A) was obtained by removing a 1-mm-thick layer from the outer surface of intact carrots, and fresh pulp (B) constituted the remainder of the peeled carrot. Additional carrots were then washed, surface-sterilized by immersion in 95% alcohol for 2 minutes, sliced longitudinally under aseptic conditions, and placed in sterile Petri dishes. Four test materials were prepared from these slices. Inoculated tissue (C) was obtained by covering the sterile slices with a heavy spore suspension of *C. fimbriata* and incubating them 6 days at 28°C. After incubation, the top 1 mm of inoculated tissue was removed for extraction. Autoclaved inoculated tissue (D) was obtained by subjecting autoclaved slices to the same treatment as in (C). Incubated tissue (E) and autoclaved incubated tissue (F) were obtained by incubating carrot slices and removing the top 1 mm of tissue as in (C).

After 3 days of incubation, no fungal growth was evident on the surface of living carrot tissue inoculated with *C. fimbriata*. After 6 days of incubation, fungal growth was barely discernible on this tissue. This was in contrast to the luxuriant growth made by the fungus on autoclaved tissue.

Preparation and activity of extracts.—Extracts of the above tissues were prepared by dropping 100 g of the tissue into 350 ml of boiling alcohol and boiling for 5 minutes. After cooling, the tissues were chopped in alcohol in a Waring Blendor. The resulting preparations were filtered through Whatman No. 2 paper on a Buchner funnel, and the filtrates collected.

Filtrates of (A) through (F) were each evaporated to dryness under reduced pressure, at temperatures not exceeding 28°C. and the residues were suspended in carrot-dextrose broth to give a concentration corresponding to 40 g of fresh carrot tissue in 50 ml carrot-dextrose broth. The suspensions were then filtered and 15-ml aliquots were placed in 50-ml Erlenmeyer flasks. The flasks were autoclaved at 15 lb pressure for 15 minutes (121°C.), cooled, inoculated with 0.1 ml of a heavy spore suspension of *C. fimbriata*, and incubated 6 days at 22°C. The media with fungus growth were filtered through previously dried and weighed Whatman No. 50 filter paper, and the papers were dried in a vacuum oven at 50°C and weighed.

Extract (C) was found to inhibit the growth of

Table 1.—Growth of C. fimbriata in media containing carrot tissue extracts

Extract added to carrot dextrose broth	% growth compared with control*
A. Peel	91
B. Pulp	166
C. Pulp tissue inoculated with *C. fimbriata* and incubated 6 days	4
D. Pulp tissue autoclaved, inoculated, and incubated	229
E. Pulp tissue held 6 days	212
F. Pulp tissue autoclaved and held 6 days	249

* Based on dry weight of mycelium produced in carrot-dextrose broth.

C. fimbriata markedly (Table 1). Since extracts (B), (D), (E), and (F) were not inhibitory, it would suggest that the inhibitor was produced through the metabolic interaction of living carrot root tissue and the fungus.

Chromatography of extracts.—Extracts (A) through (F) were evaporated under reduced pressure to a concentration corresponding to 40 g of fresh carrot tissue per 100 ml 95% alcohol. Approximately 50 μl of each extract was applied as spots to Whatman No. 1 filter paper, and the papers were developed using *n*-butanol, acetic acid, and water (4:1:1 v/v) as the descending solvent. A high concentration of chlorogenic acid was found in extract (C). A lower concentration of the acid was found in extract (A), and a trace in extract (E). A compound having an R_F value of 0.84, and appearing yellow after spraying with diazotized sulfanilic acid and purple after spraying with aqueous ferric chloride solution, was found only in extract (C). This compound was eluted from chromatograms with 95% alcohol, and the ultraviolet absorption spectrum of the eluate was determined with a Beckman model DU spectrophotometer. The eluate exhibited 2 absorption maxima, one at 267 mμ and one at 301 mμ, and an absorption minimum at 285 mμ.

Location of fungitoxic compound on chromatograms.—About 2.5 ml of extract (C) was streaked on each of two $18\frac{3}{4}\times22\frac{1}{2}$-in. sheets of Whatman No. 1 paper with a micropipette. The papers were developed with *n*-butanol, acetic acid, and water (4:1:1 v/v), and dried for 48 hours. Control strips were then sprayed with diazotized sulfanilic acid. Fourteen bands having the following R_F values were then cut from the chromatograms and eluted overnight with 95% alcohol in Goldfisch extractors: 0, the origin; 1, .01–.05; 2, .05–.08; 3, .08–.09; 4, .09–.13; 5, .13–.17; 6, .17–.21; 7, .21–.26; 8, .26–.31; 9, .31–.37; 10 (chlorogenic acid), .37–.49; 11, .64–.71; 12, .71–.77; 13, .77–.86; 14, .86–92. The eluates were concentrated to 5 ml, and 1 ml of each eluate was applied to standard antibiotic assay disks. The disks were autoclaved 15 minutes at 15 lb pressure (121°C), and then placed in sterile Petri dishes on Difco potato-dextrose agar uniformly seeded with spores of *C. fimbriata* (10 ml heavy spore suspension per 100 ml agar). The Petri dishes were incubated at room temperature for 7 days, at the end of which time, zones of inhibition were seen only around the disks containing the eluate from band 13. The ultraviolet absorption spectrum of this eluate was identical to that of the compound found only in extract (C). Paper chromatographic analysis of the eluate revealed the presence of a single spot having the same R_F and color reactions with diazotized sulfanilic acid and ferric chloride as those reported earlier for the compound in extract (C).

Isolation of fungitoxic compounds.—Extract (C) was evaporated to dryness under reduced pressure, the residue suspended in 100 ml of water, and the suspension shaken with four 40-ml portions of A.R. grade ether. The ether fractions were combined and the ether was removed by evaporation over water under reduced pressure. The resulting emulsion was then extracted with four 40-ml portions of purified Skelly Solve B. The Skelly Solve B. fractions were combined and evaporated to dryness in a rotary-type evaporator. Chromatograms showed that the residue from the Skelly Solve B fraction contained carotenes and the fungitoxic compound. The residue was dissolved in 95% alcohol, activated charcoal was added, and upon filtration a carotene-free solution remained. Water was then added to the alcoholic solution to the point of incipient cloudiness, and the compound crystallized out as plates after standing 2 days at 5°C. It was recrystallized from aqueous alcohol and dried over anhydrous calcium chloride. M.P. 75.7°C, $[\alpha]^{25}D=-51.8$.

Elemental analysis.—Calculated for $C_{11}H_{12}O_4$: C, 63.5%; H, 5.77%; O, 30.75%. Found C, 63.4%; H, 5.86%; O, 30.75%. The calculated molecular weight for $C_{11}H_{12}O_4$ is 208. The molecular weight of the compound as determined by the Rast-Soltys method is 218.5.

Inhibitory activity of fungitoxic compound.—Twenty mg of the compound were dissolved in 2 ml of 95% alcohol, and the solution was added to 100 ml of carrot dextrose broth. The concentration of the compound in this solution was $1\times10^{-3}M$. Dilutions of this solution were made to give concentrations of 5×10^{-4}, 1×10^{-4}, 5×10^{-5}, and $1\times10^{-5}M$. Corresponding dilutions were made of a control solution containing 2 ml of 95% alcohol in 100 ml of carrot-dextrose broth. Three 15-ml aliquots of each solution were pipetted into 50-ml flasks, the flasks were autoclaved 15 minutes at 15 lb pressure (121°C.), cooled and inoculated with 0.1 ml of a heavy spore suspension of the fungus under study. The inoculated flasks were incubated at room temperature, the length of incubation depending on the rapidity of the growth of the fungus under study in the control solutions. *C. fimbriata* was incubated 6 days, Race 1 *Helminthosporium carbonum* Ullstrup 5 days, and *Stemphylium radicinum* (Meier, Drechs. & Eddy) Neerg 4 days. After incubation the dry weight of mycelium was determined as described earlier.

The results are summarized in Table 2. The compound isolated from extract (C), at concentrations of 5×10^{-4} and $1\times10^{-3}M$, inhibited growth of the three fungi tested more than 50% in comparison with growth in the control solutions.

Penetration of carrot tissue by the fungus.—Carrot root slices were inoculated with *C. fimbriata*, and sections perpendicular to the inoculated surface were cut and studied 24, 48, and 72 hours after inoculation. The sections were stained with a 0.001% solution of Janus Green B for 20–30 minutes, and then washed and mounted in physiological saline solution. Sections were examined with a microscope under 900× magnification. Twenty-four hours after inoculation, spores were seen on the inoculated surface of the carrot slice. Many of these spores had begun to germinate, though none appeared to have hyphae that penetrated undamaged carrot cells. Forty-eight hours after inoculation, hyphae were observed within carrot cells. Some of the hyphae were observed one cell layer below the

Table 2.—Growth of fungi in media containing the fungitoxic compound

Organism	Concentration of compounds[a]	% growth[b]
C. fimbriata	$1\times10^{-3}M$	5
	$5\times10^{-4}M$	34
	$1\times10^{-4}M$	69
	$5\times10^{-5}M$	128
H. carbonum	$1\times10^{-3}M$	4
	$5\times10^{-4}M$	24
	$1\times10^{-4}M$	78
	$5\times10^{-5}M$	73
S. radicinum	$1\times10^{-3}M$	1
	$5\times10^{-4}M$	40
	$1\times10^{-4}M$	84
	$5\times10^{-5}M$	80

[a] Compound is dissolved in alcohol and added to carrot-dextrose broth.

[b] Based on dry weight of mycelium produced in carrot-dextrose broth containing an amount of alcohol equivalent to that used to dissolve the compound.

inoculated surface and others were found in cells 3–4 cell layers below. The hyphae were twisted and gnarled and often highly branched. This is in contrast to the straight, thin, unbranched hyphae found growing aerially on the surface of autoclaved carrot tissue or on other solid media. Seventy-two hours after inoculation a dense area appeared below the inoculated surface. This area was dark in color and apparently consisted of collapsed carrot cells and fungal hyphae. Rarely have hyphae been observed below this dark area. Traces of the fungitoxic compound were evident in extracts of the inoculated tissue at 48 hours, and considerable quantities could be detected at 72 hours.

Specificity of the interaction of carrot root tissue and C. fimbriata.—Carrot slices were inoculated with either *H. carbonum*, a pathogen of corn, or *S. radicinum*, a pathogen of carrots. The tissues were harvested and extracted, and the extracts were examined as described with *C. fimbriata*. In addition, white potato tuber slices and sweet potato slices were inoculated with *C. fimbriata* and studied.

The fungitoxic compound, produced when carrot root tissue was inoculated with *C. fimbriata*, was not found in any of the above extracts. A high concentration of chlorogenic acid, caffeic acid, and derivatives of these compounds was found in all of these extracts. A new fungitoxic compound, as yet unidentified, was found in the extract prepared from white potato tuber slices that had been inoculated with *C. fimbriata*.

Partial characterization of fungitoxic compound.—The fungitoxic compound was dissolved in 95% alcohol, spotted on Whatman No. 1 filter paper, and chromatographed as described earlier. After drying, chromatograms were sprayed with diazotized sulfanilic acid, aqueous ferric chloride, alcoholic aluminum chloride, phloroglucinol in HCl, bromcresol green, ninhydrin, and ammoniacal silver nitrate. Tests for the following functional groups were performed: ester (5, p. 122), alkoxyl (5, p. 116), methylene (2, p. 221), enol (2, p. 139), and methylene dioxy. To test for the presence of a methylene dioxy group, the compound was heated with concentrated sulfuric acid to 100°C, and a small amount of chromotropic acid was then added. A positive reaction is the production of a violet color.

The ultraviolet absorption spectra, between 250 mμ and 350 mμ, of the fungitoxic compound in 95% alcohol and in 0.1*N* sodium hydroxide were determined on a Beckman model DU spectrophotometer. The infrared and near-infrared absorption spectra were respectively determined on the Baird double-beam recording spectrophotometer between 2 and 16 μ and the Cary recording spectrophotometer between 1 and 2.6 μ.

Fifty-seven mg of the compound was dissolved in 15 ml of 0.5*N* sodium hydroxide and the solution refluxed for 6 hours. The solution was then cooled, acidified to pH 2 with 1*N* hydrochloric acid, and extracted with three 10-ml portions of ether. The ether and water fractions were each evaporated to dryness in a rotary-type evaporator, and the residues were dissolved in 95% alcohol. The ultraviolet absorption spectra of the two fractions were determined, and the fractions were chromatographed as described earlier.

The fungitoxic compound gave a positive reaction with diazotized sulfanilic acid (yellow), aqueous ferric chloride (purple), alcoholic aluminum chloride (fluorescence), phloroglucinol in hydrochloric acid (pale violet), bromcresol green (faint yellow). The compound gave no color reaction with ammoniacal silver nitrate and ninhydrin. Tests for alkoxyl, methylene, and methylene dioxy groups were negative, whereas the compound gave a doubtful test for an enol and a positive test for an ester.

The compound exhibited two absorption maxima in the ultraviolet, a primary peak at 267 mμ (ϵ 11390) and a secondary peak at 301 mμ (ϵ 4550). The absorption maxima in 0.1*N* sodium hydroxide were 266 mμ (ϵ 8690) and 330 mμ (ϵ 6970).

The compound exhibited strong absorption peaks at 1670 cm^{-1}, and 1245 cm^{-1}, which have respectively been assigned to a hydrogen-bonded carbonyl stretching vibration, an aromatic ring of deformation vibration, and a carbon-oxygen stretching vibration. It also exhibited a large peak at 2.27 μ, which is characteristic of methyl groups.

When the fungitoxic compound was refluxed with 0.5*N* sodium hydroxide, and the ether and water fractions of the acidified solution chromatographed, 2 compounds were apparent in the ether fraction. One was the fungitoxic compound, the other a new compound giving a red color when sprayed with diazotized sulfanilic acid and a violet color with ferric chloride. The ultraviolet absorption spectrum of the new component was identical to that of the fungitoxic compound.

The fungitoxic compound is soluble in 5% sodium hydroxide, concentrated sulfuric acid, ether, carbon tetrachloride, hexane, or 95% alcohol, and is insoluble in water or 5% sodium bicarbonate.

Discussion.—A fungitoxic compound has been found in carrot root tissue penetrated by *C. fimbriata*, and

the production of this compound appears to be dependent on the interaction of living carrot tissue and fungus. The possibility that this compound could be produced either by the fungus itself or by an autolytic process in the carrot is minimized by the fact that it appears only in the extract of living inoculated tissue (C). This compound could not be found chromatographically in extracts prepared from carrot root slices inoculated with *H. carbonum*, a corn pathogen, or *S. radicinum*, a pathogen of carrots in storage; in extracts prepared from sweet potato slices, the normal host, inoculated with *C. fimbriata;* or in extracts prepared from white potato tuber slices inoculated with *C. fimbriata*.

The largest yield of the compound obtained has been 410 mg from 664 g of inoculated carrot tissue. If one assumes this to be the concentration of compound in the inoculated carrot slice, and using the calculated molecular weight of 208, the concentration of compound in the inhibitory carrot tissue extract would be at least $2.5\times10^{-3}M$. A concentration of $1\times10^{-3}M$ of the compound in carrot-dextrose broth was found to inhibit the growth of the fungus 95% in comparison with growth on carrot dextrose broth alone. This compound, therefore, could account entirely for the inability of *C. fimbriata* to make good growth in the extract of living inoculated carrot tissue.

Although chlorogenic acid does increase in carrot tissue after inoculation, it seems to have little effect on the growth of the fungus. Chlorogenic acid and other phenolic compounds are produced in various plants both in response to pathogens and to nonpathogens (3, 6). It has been suggested that this is a nonspecific host reaction. The present work seems to substantiate this suggestion.

Chemical tests indicate that the fungitoxic compound may be phenolic in nature and possess a double bond, probably the carboxyl group of an ester linkage, in conjugation with a benzene ring. It also appears that the fungitoxic compound possesses a hydroxyl group ortho to an esterified carboxyl group. A coumaran-type structure is proposed.

The preparation of derivatives and further characterization of the compound is now being undertaken.—Department of Biochemistry and Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana.

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A QUICK METHOD OF PREPARING BARLEY EMBRYOS FOR LOOSE SMUT EXAMINATION

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SUMMARY

A 90-minute method of preparing barley embryo whole mounts for loose smut determinations was developed. The steps include extraction (45–60 minutes) in a boiling solution of 5% sodium hydroxide and 14% commercial liquid glass (sodium silicate) to which a small quantity of detergent is added, separation (5 minutes) on screens, separation (5–15 minutes) by centrifugation on 50% liquid glass, and clearing (10 minutes) in boiling lactophenol. The times listed include normal handling between steps. Undamaged embryos of high clarity are obtained. The mycelium of the smut fungus is reddish-brown, and can be detected with a 15× dissecting microscope.

Introduction.—A heavy outbreak of barley loose smut (*Ustilago nuda* (Jens.) Rostr.) in North Dakota in 1959 stimulated interest in developing a rapid index of barley seed for smut infection.

Simmonds (5) first described success with whole embryo mounts for loose smut determinations, and Russell (3) and Russell and Popp (4) showed that such embryo tests have a very high correlation with field and greenhouse indexes.

Popp (1) pointed out that the test has certain limitations for loose smut of wheat, since numbers of smutted plants are affected not only by percentage of infection in the seed but also by host variety and physiologic race of the smut fungus. For loose smut of barley, however, Russell and Popp (4) found no indication that these factors interfere with close agreement between embryo and growth tests on naturally inoculated seed.

In an unpublished report, Russell described a modification of Simmond's whole-mount technique, which is used by the Canada Department of Agriculture for indexing barley seed. Briefly, it consists of placing seed in 5% sodium hydroxide for 15 hours, partially separating embryos from the rest of the seed by screening, completing separation by picking embryos

out with a medicine dropper, and clearing embryos in ethanol for 1 day and lactophenol for 2 days. The procedure is estimated to take about 2 hours per sample, spread over a 4-day period.

Popp (2) has described a faster method of preparing wheat and barley grain for loose smut examination. In this method, hulls are separated with a Waring Blendor and embryos are extracted by boiling kernels 30 minutes at 5 lb. pressure in a solution of sodium hydroxide, sodium silicate (liquid or water glass), and detergent. Embryos are then separated by flotation with sodium silicate, and cleared by heating 45 minutes at 5 pounds in ethanol and sodium hydroxide, washing 15 minutes in water, reheating 1 minute at 5 pounds in ethanol and glacial acetic acid, and again heating 1 minute at 5 pounds in lactic acid. Staining is accomplished by boiling the embryos 1 minute at 5 pounds in acetic acid and trypan blue, and then removing the excess stain by heating 1 minute at 5 pounds in lactic acid. This process is completed in only a few hours but requires considerable manipulation. Very clear embryos with conspicuous mycelium are obtained.

In developing a test for use in North Dakota, a combination of these techniques along with certain modifications was found to require only 90 minutes or less to prepare embryos for examination. All parts of the embryos are clear and are morphologically intact, while smut mycelium is easily seen and can be detected with 15× magnification on a dissecting microscope.

Materials and methods.—*Extraction of embryos.*—For a practical test, 600–700 barley kernels are boiled in 500 ml of water solution containing 25 g (5%) sodium hydroxide, 70 ml (14%) liquid glass, and one drop of liquid detergent. This number of barley kernels ensures that over 500 embryos will be available for examination. A 1000-ml beaker is a suitable container, and the kernels are boiled vigorously until they become a gelatinous mass. Occasional stirring promotes uniform results, and water added to replace that boiled away prevents the mixture from becoming too viscous. Boiling time is 45–60 minutes, depending largely on the heat applied. Boiling at 5–15 lb pressure was not appreciably faster, and prevented close observations of the process.

Greater concentrations of sodium hydroxide than 5% do not accelerate separation and lower concentrations are less effective and delay development of the gelatinous stage. The 14% liquid glass protects the embryos from physical damage, and the drop of detergent prevents foaming.

Separation of embryos.—The gelatinous mass is poured while still hot onto a series of screens of 4, 6, 8, and 14 meshes per sq. in. When warm water is sprayed gently on the mass the embryos and lighter hulls are quickly carried to the lowest screen, while the endosperms and some hulls are caught above. The contents of the lowest screen are washed into a pan, poured into a beaker, and condensed by decanting excess water.

The embryos are separated from hulls and other fine debris by centrifugal force. An International Clinical Centrifuge with a 4-bucket head using 50-ml tubes is run at 4500 rpm. The tubes are filled about ¾ full with a 50% aqueous liquid glass solution (also effective are saturated sucrose, glucose, or sodium chloride solutions), and the contents from the bottom screen are placed on top of the solution. Separation time varies with the thickness of the sample, but is usually about 5–15 minutes. The embryos form a layer at the top of the 50% liquid glass, while the hulls and other debris are deposited at the bottoms of the tubes. The embryos are easily poured off, and after being washed in 2 changes of water are ready for clearing.

Popp (2) used flotation with liquid glass to separate embryos from other residues. Flotation, however, appears not to separate embryos from hulls, whereas centrifugation gives virtually complete separation. Popp removed the hulls in a Waring Blendor. It is likely, though, that the centrifuge would be superior to the blender, for the embryos are not damaged by the centrifuge whereas they may be by the blender.

Clearing.—Excess water is poured from the embryos, which are then placed in Petri dishes. Lactophenol (1:1:1:5 phenol (melted), glycerine, lactic acid, and distilled water) is added until the dishes are ½–¾ full, and the contents are boiled several minutes, until the water has mostly evaporated and the remaining fluid commences to fume. The dishes are then quickly removed from the heat, and when cooled are ready for examination. The preparations must be removed when fuming starts or they may be destroyed. If embryos of less clarity are desired, they should be removed after a shorter boiling time.

Examination.—The cooled embryos are in a viscous solution and remain relatively stationary when the dishes are handled. They are exceptionally clear under the microscope, and the mycelium of the smut fungus is a striking reddish-brown network, found mainly in the scutellum.

The embryos may be examined rapidly with a dissecting microscope having 15× ocular and 1× objective lenses. Infected embryos show the characteristic coloring in the scutella and may be checked under higher magnification if infection is in doubt. Grooved dishes or gridded designs on the Petri dishes speed the counting.

Discussion.—The method described is fast and relatively simple. A maximum of 90 minutes is required to prepare embryos for examination, and undamaged embryos of high clarity are obtained.

A somewhat slower and less critical variation may be more suitable at some facilities. The original extraction may be run at room temperature for about a day, or until the kernels are a gelatinous mass. Water may occasionally be added to keep viscosity fairly low. With this variation, centrifugation is often unnecessary.

The clearing may be done by keeping the embryos in lactophenol in a drying oven at 60°C or higher until the water is evaporated from the solution (several hours). Embryos kept in ethanol for 1 day and lactophenol for 3 days at room temperature were not as

clear as embryos boiled or put in the oven for several hours.—Department of Plant Pathology, North Dakota Agricultural College, Fargo.

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EFFECT OF SOFT ROT BACTERIA AND PECTOLYTIC ENZYMES ON ELECTRICAL CONDUCTANCE OF WITLOOF CHICORY TISSUE

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SUMMARY

Low-frequency alternating current of 1 kc/second was used to measure the electrical conductance of witloof chicory leaves infected by soft rot bacteria. Inoculation with living cells or culture filtrates of *Erwinia* sp. and *Pseudomonas* sp. resulted in increased tissue conductance, with soft rot and increase in conductance being greater from *Erwinia* sp. than from *Pseudomonas* sp. Tissue conductance increased before symptoms developed and in advance of the margin of visible lesions. Tissue injury was the result of the action of pectolytic enzymes produced by the pathogens, for a mutant nonpectolytic strain of *P. marginalis* caused neither soft rot nor conductance changes.

It has been shown that there is an increase in the electrical conductance of tissue following injury from various factors, such as electricity (13, 14), freezing (5, 12), chilling (11), heat (12), or chemicals (9, 12). Recently Greenham and Müller (10) studied the conductance changes in potato tubers injured by infection with *Phytophthora infestans* and *Pythium* sp., and Drachovska and Sandera (4) used the increased conductance in diseased or damaged tissue for diagnosing sugar beet yellows and evaluating *Cercospora beticola* infection. Since similar studies have not been found concerning bacterial diseases of plants, the present paper reports on changes in the electrical conductance of leaf midrib tissue of witloof chicory (*Cichorium intybus* L.) caused by soft rot bacteria and their pectolytic enzymes.

Materials and methods.—Electrical conductance was measured with a battery-operated impedance bridge (General Radio Type 650-A) and earphones (Brush Electronics Type BA-200) using a low-frequency alternating current of 1 kilocycle per second. The electrode probes, modified after Greenham and Cole (9), consisted of 24-gauge nichrome wire mounted in glass capillary tubes enclosed in a tubular plastic jacket and sealed with white beeswax. The electrodes were set parallel, 3 mm apart, with a penetration depth of 3 mm. For conductance readings, each leaf was placed horizontally with its adaxial surface up, and the probe was inserted perpendicularly into the leaf with one electrode on each side of the longitudinal axis of the midrib. Readings, taken at room temperature (21–26°), were made in resistance (ohms) and converted to conductance (mhos), the reciprocal of resistance.

Two soft rot bacteria, isolated in 1958, from natural infections of witloof chicory, were used. One, a *Pseudomonas* sp., caused a slow, limited, brown soft rot similar to that caused by *P. marginalis* (6), whereas the other, an *Erwinia* sp., caused a rapid, extensive, light-colored soft rot. Both organisms in culture filtrates produced pectolytic enzymes as shown by maceration of lettuce butt tissue and viscosity reduction of pectin solutions (Friedman, unpublished). The control was an ultraviolet-light-induced mutant strain (M4) of the soft rot bacterium *P. marginalis*. As a result of irradiation this strain had lost its pathogenicity for witloof chicory and its ability to produce pectolytic enzymes in culture (7).

Individual witloof leaves were inoculated by needle, in the adaxial surface of the midrib about 1 cm from the cut base, with 24- or 48-hour-old cultures of each of the 3 bacterial isolates grown on yeast-extract nutrient agar. Sterile filtrates of each of the 3 isolates prepared from 4-day-old lettuce broth cultures by passage through Seitz filter pads were adjusted to pH 8.0 with *N* NaOH after the addition of 100 ppm of streptomycin. The details have been reported (2). Cut leaves were placed, base down, to a depth of about 1 cm in a 100-ml beaker containing the filtrate. Leaves inoculated with bacteria were placed in moist chambers, whereas leaves standing in filtrates were left exposed to the open air. All were held at 21–26°C. In an additional experiment, made to determine why leaf tissue lesions resulting from inoculation with bacteria-free culture filtrates had greater conductance than lesions resulting from inoculation with bacteria, cells of *P. marginalis* M4, *Pseudomonas* sp., or *Erwinia* sp. were inoculated 5 mm from the base of 72 witloof leaves. After 7 hours in moist chambers, half the leaves were removed and placed upright to a depth of about 5 mm in sterile lettuce broth containing 100 ppm streptomycin. Conductance readings were made 17 hours later on all leaves at the site of inoculation.

Results.—Fig. 1 shows the changes in electrical conductance of midrib tissue of witloof chicory at the site of inoculation with living cells and culture filtrates of *Erwinia* sp., *Pseudomonas* sp., and *P. marginalis* M4.

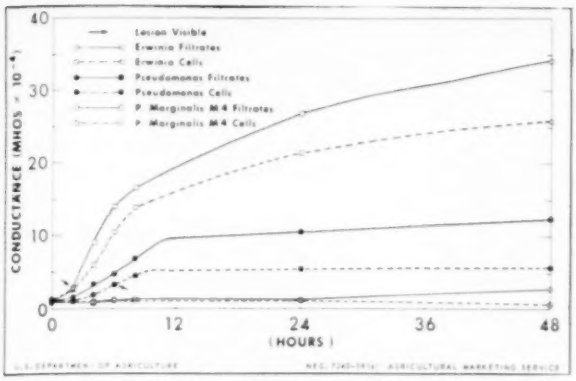

Fig. 1. Electrical conductance of witloof chicory tissue at site of inoculation with cells and culture filtrates of soft rot bacteria.

The results represent conductance readings on 378 leaves (3 cultures plus 3 filtrates $\times$ 3 leaves $\times$ 7 time periods $\times$ 3 replications). The pathogens or their filtrates markedly increased the conductance of affected tissue; the avirulent *P. marginalis* M4 caused no conductance changes. *Erwinia* sp. increased conductance more than did *Pseudomonas* sp. *Erwinia* sp. produced a visible lesion in 2 hours, and *Pseudomonas* sp. took 6 hours. Before the visible lesions developed, an increase was observed in electrical conductance of inoculated tissue (Fig. 1).

Fig. 2-A,B shows conductance readings made after 8 hours at the site of inoculation with bacterial cells and cell-free filtrates and at 5-mm intervals therefrom. Each point on these 6 curves represents an average of 9 conductance readings on each of 54 leaves. The arrows on the figure represent the extent of the visible lesion from the point of inoculation. The figure illustrates, for example, that conductance was increased over that of healthy tissue about 5–7 mm in advance of *Erwinia* lesions and about 4–5 mm in advance of *Pseudomonas* lesions. In an additional experiment, using 63 leaves, conductance readings were made at 2-mm intervals from the margin of 8-hour-old lesions produced by bacterial cultures. Conductance was increased about 12 mm in advance of *Erwinia* lesions and about 6 mm in advance of *Pseudomonas* lesions.

During the experiment 1134 conductance readings were taken on leaves inoculated with cells or cell-free filtrates of *P. marginalis* M4, but in no case did any lesion develop. About 99% of the readings ranged from 0.8×10^{-4} to 1.3×10^{-4} mhos, the mean being 1.0×10^{-4} mhos.

In the experiment to determine why filtrate-produced lesions had greater conductance than bacteria-produced lesions, the conductance of leaves held for the entire 24-hour period in moist chambers was respectively 0.8×10^{-4}, 1.9×10^{-4}, and 11.9×10^{-4} mhos for *P. marginalis* M4, *Pseudomonas* sp., and *Erwinia* sp. The respective conductance of comparable leaves transferred to lettuce broth at the end of 7 hours was 1.0×10^{-4}, 5.3×10^{-4}, and 17.6×10^{-4} mhos.

Discussion.—The increase in electrical conductance of plant tissue following injury or death is attributed to increased permeability of the semipermeable membranes of tissue cells permitting leakage of ions to the intercellular spaces (3, 12, 13, 14). Various inorganic ions (especially potassium), amino acids, and peptides have been reported to escape from injured cells (8, 11). Extracellular pectic substances are believed to exist in bound form, such as calcium and magnesium pectates (1). Consequently, aside from the increase of electrolytes in intercellular spaces resulting from exosmosis from injured cells, there is the possibility that the hydrolysis of extracellular pectic substances by pectolytic enzymes releases bound ions with a further increase in tissue conductance.

Apparently any cellular injury, as well as injury from disease caused by fungi or virus, increases the electrical conductance of tissue. The present paper shows that the electrical conductance in witloof chicory midrib tissue is increased by bacterial soft rot caused by *Pseudomonas* sp. or *Erwinia* sp. or by their cell-free culture filtrates containing pectolytic enzymes. The increase in conductance parallels the severity of tissue injury. The results also show that conductance increases before a lesion is visible and also in apparently normal tissue in advance of the margin of the visible lesion. Studying cell permeability changes by plasmolytic methods, Thatcher (15) reported that *Botrytis* and *Sclerotinia* infections of celery petioles increased permeability in advance of the margin of the visible lesions. The tissue maceration and conductance increase undoubtedly result from the production of pectolytic enzymes by the 2 soft rot bacterial pathogens, for these changes are not observed following inoculation with *P. marginalis* M4, which causes no disease and produces no pectolytic enzymes (7).

Since the concentration of pectolytic enzymes decreases as filtrates stand at room temperature, it was expected that witloof chicory tissue inoculated with bacteria would show a greater conductance increase than tissue exposed to filtrates. The reverse was observed (Fig. 1). The explanation appears to be found in the experiment on the effect of standing leaves inoculated with the bacterial cultures in lettuce broth.

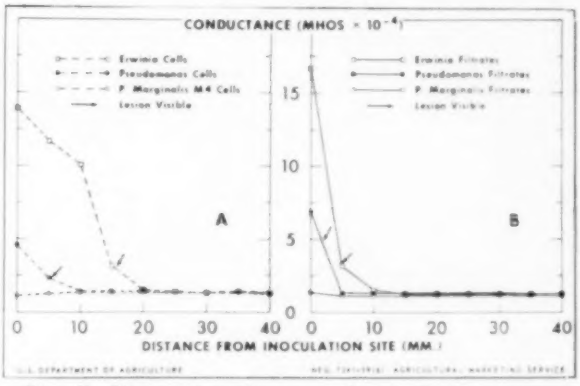

Fig. 2. Electrical conductance of witloof chicory tissues taken at 5-mm intervals from inoculation site 8 hours after inoculation with cells (**A**) or culture filtrates (**B**) of soft rot bacteria.

The greater conductance of these leaves than those held in moist chambers apparently results from uptake by macerated tissues of electrolytes from the lettuce broth.

In an advanced stage of soft rot (24 hours after inoculation, or later) witloof chicory leaves infected by *Erwinia* sp. are completely macerated and collapsed, and can be readily distinguished from leaves bearing the smaller dark soft rot lesions caused by *Pseudomonas* sp. In early stages of infection, however, lesions caused by *Erwinia sp.* can hardly be distinguished from those caused by *Pseudomonas* sp. Then the two bacteria might possibly be distinguished in natural infections by the large difference in electrical conductance (Fig. 1). Similar considerations may possibly apply in diagnostic separation of other diseases that resemble each other on a given host and cannot be distinguished readily, especially under field conditions. —Market Pathology Laboratory, U. S. Department of Agriculture, New York 14.

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GERMINATION OF OOSPORES OF APHANOMYCES EUTEICHES EMBEDDED IN PLANT DEBRIS

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SUMMARY

Oospores of *Aphanomyces euteiches* embedded in plant debris particles isolated from infested soils germinated in preparations of horse-dung infusion and pea-sand leachate. Germination of oospores also occurred in cellulose casings buried next to roots of peas, soybeans, beans, and sweet corn. Significantly greater numbers of oospores germinated next to roots of peas than next to roots of other plants. The percentages of oospores that germinated ranged from 2 to 40. Germination of oospores occurred by production of germ sporangia and zoospores. Seven single-zoospore isolates were obtained from germinated oospores. These were shown to complete the full life cycle in culture and to be pathogenic to pea plants. Differences in pathogenicity and in cultural characters were noted among the 7 isolates. The fact that large numbers of oospores embedded in plant debris particles in infested soil are viable and germinate to produce zoospores strongly indicates that oospores of *A. euteiches* are the means by which the pathogen is maintained in soil in the absence of host plants. Establishment of a pathogenic culture from a single zoospore of *A. euteiches* lends support to the contention that zoospores are the ultimate infective agents. This information may also elucidate to some extent the genetics of the organism.

The probable importance of oospores of *Aphanomyces euteiches* Drechs. to survival in soil between crops of the host plant, garden pea, *Pisum sativum*, has been suggested by several workers (3, 4, 6). Germination of oospores isolated from infested soil, however, has not heretofore been reported. A method for microscopic detection of oospores embedded in plant debris particles has been described (1). This paper reports on methods of germinating these oospores, their manner of germination, and the pathogenicity of single-zoospore cultures of *A. euteiches* obtained from germinated oospores.

Materials and methods.—Field soils naturally infested with *A. euteiches* and greenhouse soils artificially infested with the pathogen were supplied by Dr. K. K. Kaukis, Green Giant Company, Le Sueur, Minnesota. Pure cultures of the fungus were supplied by Dr. T. H. King, University of Minnesota. Plant debris particles containing oospores were removed from the soil by

methods described earlier (1). The isolated particles were then transferred to a suitable medium for incubation. Media used were horse-dung infusion and pea-sand leachate prepared as described by Smoot et al (7), or tap water. These media were added to 16×3-mm concave microscope slides or 27×8-mm watch glasses. These were then placed on moist filter paper in Petri dishes and autoclaved 20 minutes at 15 lb steam pressure. After cooling, several debris particles containing oospores were put into each solution. In most experiments the wells in the slides or watch glasses were covered with glass cover slips. The vessels were then incubated at 22±2°C.

A modification of Kerr's (5) cellophane-bag technique was used to bring the debris particles in proximity to roots of growing plants for a time and to facilitate their later removal for examination in order to study oospore germination. Seamless regenerated cellulose tubing (wall thickness, 0.0254 mm; average pore radius, 24 Å) was cut into 7-in. lengths and boiled to remove glycerine. Plant debris particles containing oospores were placed inside the casings, and the membrane was then folded so as to bring the two open ends together. An opening was made in the sand in which plants were growing, and the folded end of the membrane was inserted in the sand until the open ends were ¼ in. above the surface. Sand and roots were then pressed tightly against the casing. Normal daily watering of the plants was continued until the membrane bags were removed. As controls, casings containing debris particles were placed in sand, sterilized soil, and unsterilized soil that contained no plants or roots.

Plants used were S-539 (Green Giant Company) peas, a strain uniformly susceptible to root rot incited by *A. euteiches;* Great Northern beans, *Phaseolus vulgaris,* U.I. 59; Bansei soybeans, *Glycine max;* and Golden Cross Bantam sweet corn, *Zea mays.* Ten seeds of each kind were planted in 4-in. pots containing either sand or soil. Plants were used for testing when they were 2–3 in. tall. The tests were made in a greenhouse at 20±2°C.

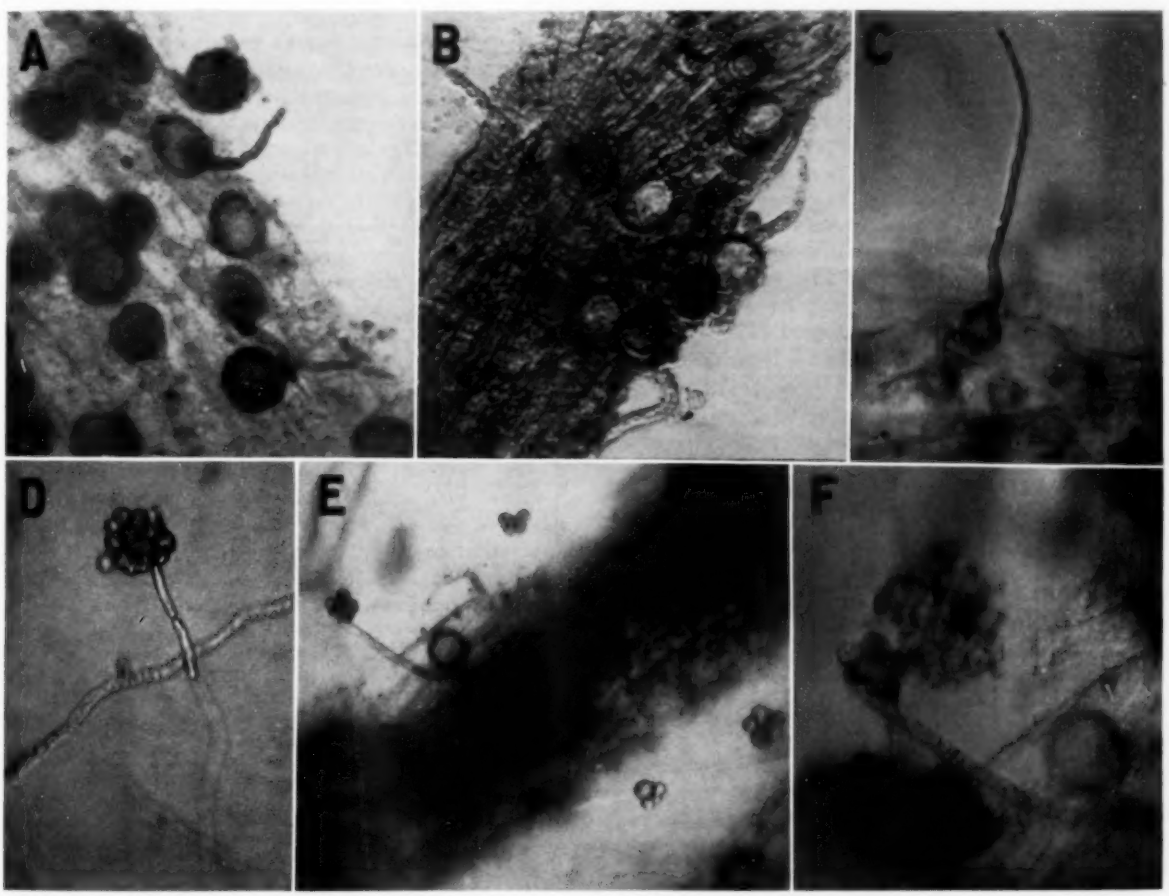

Fig. 1.—A) Oospores of *Aphanomyces euteiches* embedded in plant debris. Two oospores germinated. (Stained with methylene blue; ×500.) **B)** Oospores of *A. euteiches* in plant debris, 4 spores germinated (×500). **C)** Germinated oospore of *A. euteiches* showing initial separation of protoplasm prior to zoospore formation (×430). **D)** Asexual zoospores of *A. euteiches* encysted at a sporangial tip, with 2 zoospores still in the sporangium (×500). **E)** Germinated oospores of *A. euteiches* in plant debris, showing germ sporangia and clusters of encysted zoospores (×230). **F)** Germinated oospore of *A. euteiches* in plant debris, showing germ sporangium and cluster of encysted zoospores (×880).

Pathogenicity tests were made on isolates of *A. euteiches* obtained by isolating zoospores from germinated oospores. Field soil was placed in 4-in. pots, autoclaved 2 hours at 20 lb pressure, and planted with 10 pea seeds per pot. Plants were kept in a greenhouse at 20±2°C. During the test the plants were watered daily, keeping the soil at a high level of moisture.

Inoculum was prepared by growing cultures of *A. euteiches* in corn-kernel–water medium for 5 days and then pouring off the broth. Tap water was then added, the culture was shaken, and the water poured off. Tap water was again added, and after 36–48 hours numerous zoospores were evident. This suspension of zoospores was then poured into the pots of pea seedlings.

Results.—*Mode of oospore germination.*—Germination of oospores in horse-dung infusion and pea-sand leachate was seen in plant debris particles from 2 naturally infested field soils and 3 artificially infested greenhouse soils. In all experiments plant debris particles containing oospores were placed in both open and sealed vessels of tap water. These were examined periodically at the same times as other treatments. In no case was germination of oospores seen in water. Variable numbers of oospores germinated after 6–24 days of incubation. In one case, production of germ tubes or germ sporangia was seen in horse-dung infusion in uncovered watch glasses, but no zoospores were seen. In the laboratory, zoospores developed only from oospores in sealed vessels. Apparently an anaerobic environment is required for oospore germination by means of zoospores. The protoplasm in the oospore first became granular, the inner walls disintegrated, and germ sporangia emerged (Fig. 1-A). The sporangia grew until they attained a length 5–12 times the diameter of the oospore (Fig. 1-A,B). After several hours, the protoplasm in the germ sporangia separated into distinct units and zoospores were liberated, encysting in a cluster at the end of the germ sporangium (Fig. 1-C,D,E,F). After 2–3 hours the zoospores became active in the cyst and emerged through a papilla, swimming freely for a period. They then become quiescent, rounded-up, and under favorable conditions germinated by 1–3 germ tubes. Extensive mycelial development then occurred.

Single-zoospore cultures of the pathogen were obtained by plating-out oospore suspensions on acidified water agar and removing isolated germinated zoospores. Three single-zoospore isolates were obtained from a naturally infested field soil, and four isolates from an artificially infested greenhouse soil. These isolates produced typical oospores and zoospores of *A. euteiches* in pure culture. Pathogenicity of these cultures was tested in subsequent experiments.

Effects of the rhizosphere on germination of oospores.—Oospores in cellulose casings buried next to roots of various plants germinated at 5–10 days. Table 1 shows results of 3 experiments utilizing plants grown in sand; Table 2 shows results of one experiment with plants grown in unsterilized field soil. The data were analyzed using a chi-square test for independence in a row × column heterogeneity table (8). Analysis of the data in Table 1 showed that oospores germinated were significantly fewer in plant-free sterilized soil than in unsterilized sand or sand containing plant roots, yet germination was significantly greater in sterilized soil than in unsterilized soil. About 40% of spores germinated next to pea roots; about 33% next to roots of bean, corn, or soybean; 24% in sand; 16% in sterile soil; and 8% in unsterilized soil.

Analysis of the data in Table 2 shows essentially the same pattern of spore germination with plants grown in unsterilized field soil as with plants grown in sand, but percentages of spores germinating are considerably reduced. About 26% of oospores germinated next to pea roots, 20% next to bean and soybean, and 16% next to corn. In soil as in sand, oospore germination was significantly greater next to pea roots than next to bean, corn, or soybean roots, or in sand in which no plants were grown. No differences in oospore germination were shown between sand, sterile soil, and plant roots other than pea. In unsterilized soil, significantly fewer oospores germinated.

Pathogenicity tests.—Pathogenicity varied somewhat from test to test, but all the single-zoospore isolates of *A. euteiches* were moderately to severely pathogenic in all cases. Typical oospores were observed in the cortical tissues of all diseased roots examined. Sample roots were selected in each test, washed, and incubated in Petri dishes containing tap water. Zoosporangia and zoospores were produced in 36–48 hours.

Isolates from the naturally infested field soil seemed to be more consistently virulent than any of the other cultures tested, having an average disease class of 4 on the scale defined by Sherwood (6). Isolates from artificially infested soil were pathogenic, having an average disease class of 3. They did not show as much variability as cultures 8, 9, and 10 with an average disease class of 3. These had been maintained in artificial media for at least one year, so they might have lost some of their virulence in that time.

It is evident that the single-zoospore isolates are pathogenic and in some cases more virulent than cultures isolated from vegetative mycelium. The fact may

Table 1.—Number of oospores of A. euteiches in plant debris particles germinated to produce zoospores after 7 days in sand next to roots of various plants and in sand or soil in which no plants were grown (data from 3 experiments)

Treatment	Number germinated	Total	% germinated	χ^2
Pea	248	616	40	33.96**
Bean	166	505	33	2.23
Corn	209	611	34	6.62
Soybean	269	827	33	3.63
Sand	112	459	24	5.55
Sterile soil	36	221	16	18.32**
Unsterilized soil	33	398	8	86.11**
Total	1073	3637		

** Significant at 1% level.

Table 2.—Number of oospores of A. euteiches in plant debris particles germinated to produce zoospores after 7 days in unsterilized soil next to roots of various plants and in sand or soil in which no plants were grown

Treatment	Number germinated	Total	% germinated	χ^2
Pea	111	430	26	15.88*
Bean	96	485	20	.68
Corn	39	242	16	.98
Soybean	65	350	19	.02
Sand	44	236	19	.02
Sterile soil	57	375	15	2.88
Unsterilized soil	3	136	2	23.72**
Total	415	2254		

** Significant at 1% level.
* Significant at 5% level.

also be stressed that no sexual fusion between single-zoospore cultures is necessary for pathogenicity or completion of the life cycle of the fungus.

Discussion.—It has been shown that the number of plant debris fragments infested with oospores of *A. euteiches* has a high positive correlation with the severity of root rot of peas in soil from which the plant residue was obtained (1). This information, coupled with the fact that a large percentage of those oospores are viable and germinate to produce zoospores, strongly indicates that sexual spores in dead plant tissues provide the primary inoculum for new outbreaks of root rot of pea.

Since the oospores observed to germinate all did so by means of zoospores, both in the laboratory and in cellulose casings buried in the soil, the primary infective agent appears to be the zoospore. Moreover, establishment of a pathogenic culture from a single zoospore of *A. euteiches* lends support to the idea that zoospores are the infective agents. The behavior of a single-zoospore culture, producing the complete cycle, gives clues to the little known genetics of this fungus. Nevertheless, hybridization and recombination may occur since antheridia have been observed to arise from a different as well as the same hypha on which the oogonium is produced.

Examination of the differences in numbers of oospores germinating in sterilized and unsterilized soil (Table 1, 2) points up the fungistatic effect of unsterilized soil. Sterilization overcomes this effect to some degree, as pointed out by Dobbs, Hinson, and Bywater (2), but stimulation by roots of the host plant is much greater.

Besides the stimulatory factors exuded by plant roots, anaerobic conditions appear to be required for oospore germination by means of zoospores. These facts may be correlated with reports that root rot of pea caused by *A. euteiches* is more severe in wet seasons and in poorly drained soils (9). It seems quite probable that such conditions promote the germination of high percentages of oospores present in the soil, with the result that great numbers of zoospores are produced to promote spread of the disease.

The fact that the roots of certain immune crop plants may stimulate germination of *A. euteiches* oospores in the soil could possibly result in development of a method of reducing inoculum potential in an infested soil.—Department of Plant Pathology, Nebraska Agricultural Experiment Station, Lincoln.

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STREPTOMYCIN ABSORPTION IN DISEASED AND HEALTHY HOP TISSUES AND ITS EFFECT ON THE HOP DOWNY MILDEW ORGANISM AND MILDEW DEVELOPMENT

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The authors express gratitude to Dr. P. A. Ark for making some of the bioassays for streptomycin, and to Mr. Wm. G. Golden, Farm Advisor, Sacramento County, for help in field tests.

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SUMMARY

Spray at 1000 ppm streptomycin resulted in 200 ppm of streptomycin in leaves and 40 ppm in stems of diseased hop plants (*Humulus lupulus*) and 100 and 10 ppm, respectively, in healthy hops. Respective concentrations of 25 and 22 ppm stroptomycin in diseased leaves and stems were present 6 days after spray application. Diseased shoots maintained higher concentrations of streptomycin than did healthy shoots. Healthy shoot tips contained 5 ppm on the third day, and 1 ppm on the fifth. Systemic infections of downy mildew were not eliminated on these parts, nor were sporangia inactivated. *Pseudoperonospora humuli* on sprayed diseased shoots did not lose its ability to produce new spores. Nine to 43 ppm streptomycin within washed healthy leaves did not prevent infection, but sprayed unwashed leaves were somewhat protected. Chlorosis or symptomatic expression from streptomycin is directly related to the amount of streptomycin present within the tissue. More streptomycin in tissues resulted from fine sprays than from coarse sprays. Bioassays on sporangial and zoospore germination and the effect of zoospore motility showed distinctly more effect from zineb, copper sulfate, or Phaltan than from streptomycin, wettable sulfur, ziram, or cycloheximide.

Fig. 1.—Marginal necrosis **(A)** and chlorotic areas **(B)** expressed on healthy leaves 6 days after application of 1000 ppm streptomycin.

Studies with hops (*Humulus lupulus* L.) have shown the absorption and translocation of streptomycin (4) and the elimination by streptomycin of systemic infections of the downy mildew organism [*Pseudoperonospora humuli* (Miyabe & Takah.) Wilson] (3). In another fungus in the same genus [*Pseudoperonospora cubensis* (Berk. & Curt.) Rostov.], streptomycin at 100 ppm reduced the percentage of sporangial germination (1).

The present tests were made with the hop variety California Cluster (probably the same as the Late Cluster variety grown in the Pacific Northwest) to determine the effect of streptomycin on diseased and healthy plants and on the downy mildew pathogen.

Materials and methods.—Streptomycin sprays were applied on healthy and diseased shoots in hop yards. Individual hills were treated with sprays containing 10–10,000 ppm streptomycin; in addition, a concentration of 1000 ppm was sprayed twice on a four-acre plot. Sprays were applied when some sporulation was present on the leaves of systemically infected shoots. In the Sacramento (California) area, systemically invaded shoots can be detected when they are about ½ in. long, and at that time sporulation may also be evident. Streptomycin content was bioassayed with *Erwinia amylovora* (Burrill) Winslow et al by the method of Ark and Hildebrand (2). Eradication of the pathogen was evaluated by placing diseased shoots or leaves without fungus sporulation in closed containers above water, and examining periodically. Pathogenicity of spores was determined by inoculating detached leaves in Petri dishes. The leaves were supported on glass rods, and water was added to the dish.

Results.—Streptomycin solutions in concentrations of 10, 100, 1000, and 10,000 ppm were applied in the hop yard on April 22, 1958, to plants showing a few basal diseased shoots. Seven days after being sprayed, the leaves on healthy shoots showed striking chlorosis from 10,000 ppm, some chlorosis from 1000 ppm (Fig. 1), and no chlorosis from 100 or 10 ppm. On leaves receiving 1000 ppm, chlorosis developed as follows: large areas not very pronounced on the second day after spraying, areas smaller and more pronounced on the seventh day (Fig. 1), and areas still smaller and almost white, with minute necrotic flecks, after 3 weeks. Systemically infected basal shoots are normally stunted and have a distinct yellowish cast that clearly distinguishes them from healthy shoots. Infected shoots sprayed with higher concentrations of streptomycin changed to a pale green and could be distinguished from unsprayed diseased shoots. By 15 days, both sprayed and unsprayed diseased shoots had become necrotic or were dead, which is common where humidity is low and temperatures are high. Necrosis

Table 1.—Streptomycin in sap of healthy and diseased hop leaves and stems at various intervals after spraying with 1000 ppm Streptomycin

		Concentration (ppm) after intervals indicated (days)					
		1		2		6	
		Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Leaf	Unwashed	200[a]	200	150	200	30	7
	Washed	100	200	40	150	—	25
Stem	Unwashed	25	200	30	150	T[b]	25
	Washed	10	40	7	8	—	22

[a] The standard curve was not computed beyond 200 ppm.
[b] "T" indicates trace amount.

and death appeared to have been slightly delayed by the higher concentrations used.

Healthy shoots sprayed with 1000 ppm streptomycin were washed 7 days later and inoculated with sporangia. One shoot in 10 became diseased, in contrast with 2 in 6 unsprayed shoots. Five leaves collected 17 days after being sprayed became heavily infected when inoculated, whereas 15 uninoculated leaves remained free of disease. Diseased shoots collected 17 days after being sprayed with 1000 ppm streptomycin showed new sporulation within 11 hours of being placed in moist chambers, as did the unsprayed shoots collected at the same time. Sporangia from both sprayed and unsprayed shoots produced zoospores in abundance.

In another trial, when shoot growth from the crowns was about 12 in. long (May 20), a spray containing 1000 ppm streptomycin was applied to healthy and diseased shoots in the field. In the healthy leaves a slight chlorosis appeared in 48 hours and became pronounced in 96 hours. Table 1 compares the concentrations of streptomycin found in healthy and diseased tissues. Concentrations were extremely high in leaves in comparison with stem concentrations. The lower concentrations in healthy tissues of the leaf and stem may be a dilution resulting from rapid growth of shoots, which may be several inches per day. Incubation of diseased shoots on the first, second, fourth, sixth, and ninth days after application of 1000 ppm streptomycin, produced new sporulations. The sporangia liberated zoospores that caused infections on detached leaves in Petri dishes.

Four one-acre plots were sprayed with 1000 ppm streptomycin on April 24 and again on May 26. Half of the plot was sprayed at a pressure of 110 psi (fine spray), and half at 50 psi (coarse spray). Leaf chlorosis was greater from the fine spray. Streptomycin content of shoots 3 days after spraying was 3 ppm from coarse spray and 7 ppm from fine spray. Because infection may occur at the tip, streptomycin content was contrasted in shoots and shoot tips in the coarse-spray plot; respectively, they were 3 and 5 ppm on the third day, and 2.5 and 1 ppm on the fifth day. The leaves still contained 5 ppm on the seventh day.

Healthy shoots were sprayed in a second experiment, later in the year (July 21), with 1000 ppm streptomycin. Streptomycin concentration and leaf susceptibility to downy mildew were determined for 5 days on samples washed at time of picking and on unwashed comparison samples (Table 2). Absorption of streptomycin was not as high as from the applications on May 20th, but the residue on the unwashed samples on the third day was about the same. Unwashed leaves were somewhat protected from infection, whereas washed leaves, even though streptomycin was present in the range of 9–43 ppm, failed to show definite protection from infection.

The contention is supported that chlorosis in healthy leaves is related to streptomycin concentration; respective concentrations on the fourth and fifth days after spraying were 29 and 21 ppm in leaves with pronounced chlorosis and 17 and 12 ppm in slightly chlorotic leaves.

The effect of various chemicals on the production and germination of zoospores from sporangia was tested by placing sporangia in 3-ml microbeakers with the test chemical. The swarming period of zoospores in contact with chemicals was determined by adding 1 ml of zoospore suspension to 1 ml of chemical in microbeakers.

Streptomycin and other chemicals were compared in their effects on 1) germination of sporangia, 2) zoospore motility, 3) zoospore germination. The chemicals tested were Agri-mycin 100 (15% streptomycin; 1.5% oxytetracycline), Dithane Z-78 (zineb, 65% zinc ethylenebis[dithiocarbamate]), copper sulfate (technical grade), Flotox wettable sulfur (95% sulfur), Phaltan (50% *N*-trichloromethylthiophthalimide), Zerlate (ziram, 76% zinc dimethyldithiocarbamate), and Acti-dione (cycloheximide, 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide) at 1, 10, 100, and 200 ppm (Table 3).

1) *Germination of sporangia.*—Bioassays, using 4 replications of each chemical (except cycloheximide), 5 replications of the check, and 100 sporangia in each replication, were made, with incubation for 24 hours at 12°C. Streptomycin and wettable sulfur exhibited slight to no effect, whereas the other four compounds showed considerable reduction in germination. In another test, streptomycin at 100 and 1000 ppm com-

Table 2.—Streptomycin concentration in sap of washed and unwashed hop leaves related to their susceptibility to Pseudoperonospora humuli

	Streptomycin (ppm)		% leaves showing disease[a]		
Days	Unwashed	Washed	Unwashed	Washed	Check
0		43	60[b]	90	80
1		32	11[b]	65	100
2		11	30	80	100
3	115	9	10	80	100
4		12	30	70	80
5		12	30	100	100

[a] Ten leaves were used except in the checks, where 5 leaves were tested. Sporulation occurred on the check leaves within 6 days.
[b] Extremely light sporulation, in contrast with heavy sporulation in other inoculations.

Table 3.—Effect of streptomycin and other chemicals on production and motility of zoospores

Treatments	% sporangial germination in 24 hours at 12°C, Concentration (ppm)				Zoospore motility period (minutes) at room temperature, Concentration (ppm)			
	1	10	100	200	1	10	100	200
Streptomycin	82	78	71	65			15[a]	2.5[a]
Zineb	79[a]	10	9	11		5.5	1.5	0.75
Copper sulfate	84	2	3		60[a]	1.5[a]	1	1
Wettable sulfur	82[a]	78[a]	77[a]	78[a]				60
Phaltan	14	7	7	6	16.5[a]	6.5[a]	3.5	2
Ziram	68	32	18			13.5[a]	7	4
Cycloheximide							[a]	60[a]
Untreated:								
after 2 hours	11							
after 24 hours	82							

[a] Germination of zoospores was observed.

pletely inhibited germination for 2 hours, whereas the check showed 11%.

2) *Motility.*—Differences were considerable in the times required at room temperature to stop motility when various chemicals were added to water suspensions of zoospores. Copper sulfate at 10 ppm stopped motility in only 1½ minutes, though at 1 ppm motility was evident even after 60 minutes. Phaltan at 1 ppm stopped motility in 16½ minutes, but at 10 ppm required 6½ minutes. At 100 ppm, zineb, copper sulfate, and Phaltan stopped motility in less than 4 minutes whereas 200 ppm of streptomycin or ziram was required for the same effect. Wettable sulfur and cycloheximide failed to stop motility even after 60 minutes.

3) *Zoospore germination.*—Twenty-four-hour incubations of 1) and 2) resulted in no zoospore germination at 10 ppm zineb and at 100 ppm of copper sulfate, Phaltan, or ziram. Two hundred ppm streptomycin, wettable sulfur, or cycloheximide failed to stop zoospore germination.—Department of Plant Pathology, University of California, Davis.

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A BACTERIAL BROWN ROT OF PARSNIP ROOTS

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SUMMARY

A severe rot of parsnip roots on Long Island in 1959 was caused by a new species of bacterium. The causal agent had an optimum temperature of 18°C, indicating that the disease may have developed during the cool months when the roots were in the soil. The rot was not observed until spring harvest. The pathogen also caused a rot of artificially inoculated potato tubers and head lettuce; it did not infect carrots, onions, or radishes. The name *Pseudomonas pastinacae* n. sp. is proposed.

A rot of parsnip roots on Long Island was brought to my attention by Dr. A. F. Sherf in the early spring of 1959. Certain of the growers estimated their losses at half the crop. Parsnips in that district are grown in the summer, allowed to overwinter in the fields, and harvested in the spring. It was at digging time that the disease was first observed. No complaints of the disease had been made to the Plant Pathology Department at Cornell before 1959.

The disease.—The rot, frequently involving a large portion of the upper half of the root, was dark brown and rather firm. Only certain areas of the cortex were somewhat soft. A healthy parsnip root is more or less solid and at times stringy, and thus probably could not develop into a soft rot as potato tubers do when infected by a pathogen. When diseased roots were dried at room temperature, the lesions became very hard. In many instances the disease appeared to have progressed down from the crown, but certain lesions may have developed from an injury in the cortex on the side of the root.

Isolations and inoculation studies.—Microscopic examination of the necrotic lesions showed an abundance of bacteria. Dilution plates from the diseased tissue yielded many bacterial colonies. When such a technique is used in isolating a pathogen from rotting tissue, the colonies in the plate usually consist of the pathogen and many secondary organisms that have invaded the diseased tissue. With the rotting parsnip tissue, however, about 95% of the colonies on dilution plates appeared to be of one type. Colony diameter

varied with the number appearing in a plate but was normally about 8 mm. The colonies normally had a slight undulate margin and were uncommon in being colorless and transparent. After 3–4 days, the agar in the plates became pink to purple. The bacteria were transferred to tubes for further study.

The cut surfaces of slices of parsnips, potato tubers, and head lettuce were smeared with bacteria and placed in moist chambers at room temperature. In 24–48 hours, a definite rot appeared on the potato and lettuce slices, but the parsnips developed no rot even after a week. The experiment was repeated, and yellow globe onions, radishes, and carrots were also inoculated; again, only the lettuce and potato slices became infected.

Since the parsnip roots overwintered in the soil and were harvested in the early spring, infection probably took place at relatively low temperatures. Therefore parsnip, carrot, onion, and radish roots were inoculated by stabbing with a sharply pointed scalpel contaminated with the isolated bacteria and were incubated at 12, 15, 18, and 21°C. The injury on the parsnip roots was made both on the crown, at the circle where the leaves emerge, and on the side of the root in the cortex. After 2–3 weeks the inoculated parsnips showed the typical dark brown rot, from which the same bacterium was isolated (Fig. 1). Infection was most extensive at 15 and 18°C. Carrots, onions, and radishes were not infected at any temperature tested.

The pathogen was kept 3 months in culture and again inoculated into parsnip roots, which were incubated at the low temperatures indicated above. There appeared to have been some loss of virulence during the period in culture; at least, infection was less severe. The parsnips used, however, were older and firmer than those in the preceding test, and probably more resistant. No infection was obtained when the young sprouts emerging from the parsnip roots were inoculated by injuring them and applying the bacteria to the wounds.

As far as known, only *Erwinia carotovora* (Jones) Holland and *E. aroideae* (Town.) Holland have been reported as bacterial pathogens that rot parsnip roots (1). The bacterial pathogen under study was definitely neither of these organisms.

The pathogen.—The following description of the pathogen is based on 6 isolates from lesions on 6 different parsnip roots. Two reisolates, later added to the collection, were not carried through the entire series of tests. All isolates behaved similarly in culture.

Morphology.—The pathogen is a non-spore-forming rod occurring singly or in pairs. Rods from a 24-hour-old culture on beef-extract-peptone agar varied in length from 1 to 3.5 μ, with a mean of about 2.0 μ. The diameter was 0.5–0.8 μ. The bacteria are gram-negative and motile, with 1 polar flagellum.

Cultural characteristics.—Growth on beef-extract-peptone agar slants is moderate, filiform, and hyaline,

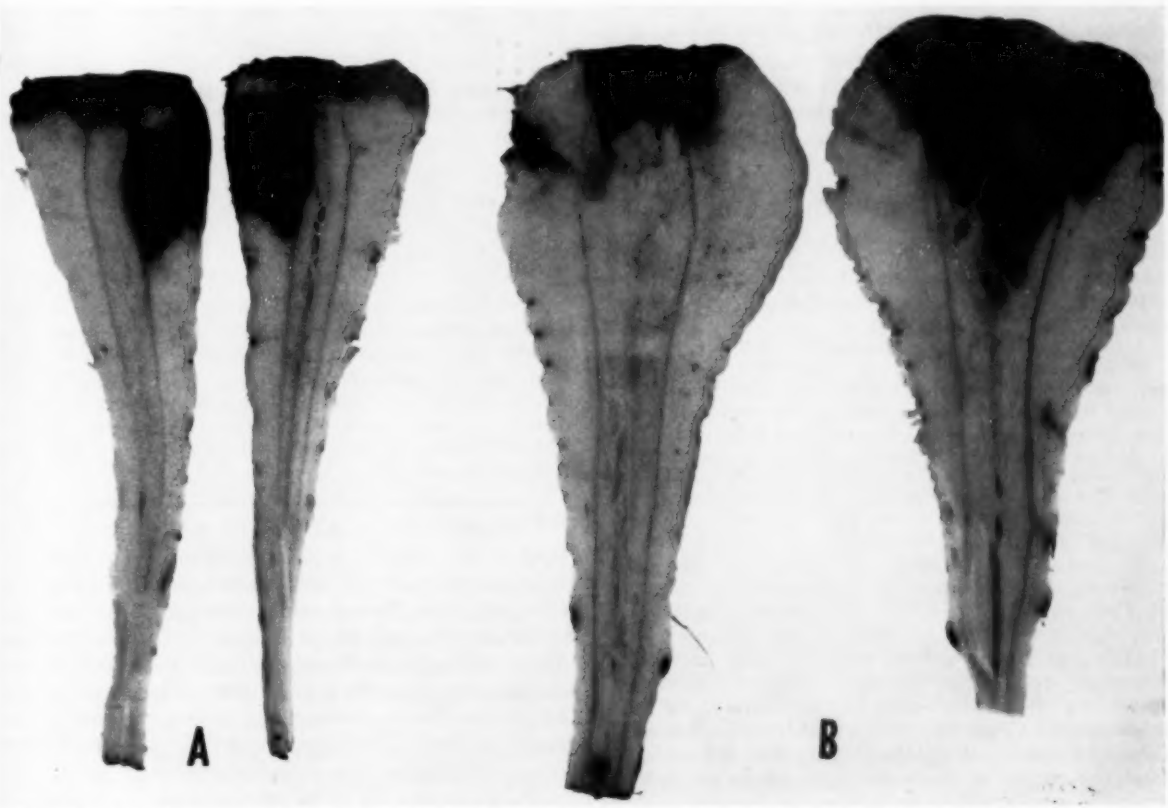

Fig. 1.—Parsnip roots artificially inoculated with *Pseudomonoas pastinacae:* **A) at 15°C; B) at 18°C.**

The consistency of the colony is butyrous. When first isolated, the bacteria impart a pink to purple color to the medium. Transfers from isolates a month old impart only a slight coloration. The coloration characteristic is restored somewhat when the pathogen is inoculated into the parsnip root and reisolated. Growth is more abundant on potato-dextrose agar than on nutrient agar and is hyaline to white, but the same color reaction takes place in the medium. Nutrient broth becomes turbid, with a pellicle that varies from light to heavy as temperature and culture age vary. The pellicle usually falls to the bottom of the tube, producing a sediment. Litmus in milk is reduced, and the milk is cleared from the surface down. The cleared serum becomes a greenish yellow. An abundant hyaline growth appears in 24 hours on Krumwiede's triple sugar agar, and the medium becomes a rose red. Clara's medium becomes turbid, with a distinct green fluorescent water-soluble pigment.

Physiological studies.—Growth in gelatin appears in 24 hours, with a rapid liquefaction following. Growth in tryptone broth is turbid, with the formation of a pellicle; no H_2S is formed. Nitrates are reduced to nitrites in a synthetic medium. The pathogen can utilize nitrates, ammonium salts, and peptone. Asparagine can be utilized as a carbon and nitrogen source. The pathogen is a strict aerobe; in deep cultures of nutrient dextrose agar, growth occurs only on the surface. It is catalase-positive.

The pathogen utilizes the following carbon sources: glucose, xylose, dulcitol, ethanol, glycerol, mannitol, and the sodium salt of citric acid. Fermentation of sucrose is delayed about a week, and growth in maltose is very slight. There is no growth in lactose or in a sodium tartrate medium. On the glycerol and mannitol media, a very yellow pellicle is formed. These tests were made in a liquid synthetic medium (Koser's medium minus the citrate and plus brom thymol blue). The sugars and ethanol were filter-sterilized; the other alcohols and organic acid salts were autoclaved.

Growth in broth occurs in 15 hours at 15–27°C, with optimum growth at 18°C, when the broth becomes very turbid with a heavy pellicle. Minimum temperature is 3°C, and maximum is above 33°C and below 37°C.

There is a slight bottom growth in broth plus 5% NaCl.

The bacterium is definitely placed in the genus *Pseudomonas* by being a gram-negative motile rod with a polar flagellum, by producing a water-soluble green fluorescent pigment, and by utilizing asparagine as a carbon and nitrogen source. Certain isolates of *P. marginalis* (Brown) Stevens produce on nutrient agar a faint pink to purple similar to that produced by the parsnip pathogen, but *P. marginalis* utilizes tartrates and has a much higher optimum temperature than does the bacterium from parsnips. Furthermore, parsnips inoculated with 2 different isolates of *P. marginalis* developed no infection. Thus the parsnip pathogen is considered to be a new undescribed species, and the name *Pseudomonas pastinacae* n. sp. is proposed. The specific name is the genitive of *Pastinaca*, the generic name of the parsnip.—Department of Plant Pathology, Cornell University, Ithaca, New York.

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EFFECT OF CULTURE SUBSTRATE ON THE VIRULENCE OF SINGLE-BASIDIOSPORE ISOLATES OF PELLICULARIA FILAMENTOSA

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SUMMARY

In comparing the effect of vitamin-amended substrates on the virulence of 3 single-basidiospore isolates of *Pellicularia filamentosa*, one isolate (Isolate 23) was significantly less virulent following culture on a vitamin-free basal medium amended with biotin and thiamine, regardless of the culture temperature. Amending media with either biotin, thiamine, or inositol decreased virulence. Two single-basidiospore selections of the original isolate (Isolate 23) did not differ from the parent in virulence after culture on vitamin-amended media, although differences between isolates occurred in the treatments that contributed to virulence. Differences occurred between the parent and progeny isolates in mycelium development after growth on vitamin-amended basal media and in the treatments that caused significantly more postemergence damping-off. A dry-weight basis of determining inoculum concentration was developed. Dry weights of inocula differed within and among isolates after growth on different media.

Introduction.—*Pellicularia filamentosa* (Pat.) Rogers, an important plant pathogen, has been investigated extensively but information is lacking on changes in morphology, metabolism, pathogenicity, and virulence of single-spore isolates of this fungus that occur during growth on certain substrates. Such changes may be a result of genetic potential, influenced by pre-inoculation environments or inoculation environments, or both. Christensen and Daly (2) and Christensen and DeVay (3) called changes that do not involve alteration of gene complement "adaptation." Other changes may result from mutations, heterokary-

osis and nuclear disassociations followed by selection or screening of biotypes. Westerdijk (10) reported that the virulence of fungi consisting of mixed biotypes changed following culture on different media. Changes in virulence of fungi arising from either mono- or multinucleate single spores or single-conidial spore isolates after culture on various media have been reported by Spilker (9), Bossley (1), and Higgins (5). In no case was it stated whether the changes resulted from environmental influences on genetic potential or from alteration of gene complements. Changes of either kind in fungi cultured on vitamin-amended substrates have not been extensively investigated, although Prasad (6) reported that one isolate of *Fusarium solani* (Mart.) App. & Wr. f. *cucurbitae* Snyd. & Hans. became more virulent following culture on vitamin-amended substrates. This paper is a report of the extent of physiologic and genetic changes that occur in single mononucleate basidiospore isolates of *P. filamentosa* and their single-spore mononucleate progenies following culture on vitamin-amended substrates. A summary has been published (8).

Materials and methods.—*Basidiospore isolation.*—Basidiospores were obtained in the manner described by Exner (4) and Sims (7). The sporulating mycelium was removed from the inside walls of 250-ml Erlenmeyer flasks and placed in test tubes containing 10 ml sterile distilled water. The resulting suspension was shaken gently to free basidiospores from basidia, and then poured through 2 sheets of lens paper to separate the mycelial fragments from the basidiospores. The filtered basidiospore suspension was diluted to 50 ml with sterile distilled water and poured serially into Petri plates containing 4% agar. Twelve to eighteen hours later, germinating basidiospores were located on the agar surface with a microscope. The area lighted by the substage light on the agar surface was decreased to a diameter just exceeding the area covered by one germinating basidiospore. After examination of the single germinating spore in the delimited light area, the objective of the microscope was racked upward and an agar disk containing the spore was cut out with a No. 1 cork borer and placed on potato-dextrose agar (PDA). Fifty single-spore isolates were thus obtained.

Media used.—A basal vitamin-free medium contained 1 g NH_3NO_3, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 15 g sucrose, and 20 g plain agar in a liter of distilled water. The medium was freed of vitamin traces after it was boiled 10 minutes with Norit A (1 g of Norit A/ 50 ml of medium). The boiled medium was filtered and placed in 125-ml vitamin-free Erlenmeyer flasks and autoclaved 15 minutes at 15 lb pressure. After autoclaving, aliquots of the medium were bioassayed with suitable assay fungi for vitamins. In no case was there evidence of vitamins.

Three vitamin-amended media were made by adding either 5 μg of biotin, 150 μg of thiamine, or 2 mg of inositol to a liter of the vitamin-free basal medium. A fourth vitamin-amended medium was made by adding 5 μg of biotin and 150 μg of thiamine to a liter of the vitamin-free basal medium.

Pathogenicity and virulence tests.—The relative pathogenicity and virulence of each isolate were obtained by first culturing each isolate 7 days on Difco PDA in Petri dishes, grinding the agar and mycelium 20 seconds in a Waring Blendor and mixing the agar-mycelial suspension at the rate of 3 Petri plates of agar-mycelial suspension to 1 gal. of steamed soil. The mixed infested soil was placed in Titelok ½-gal. containers and incubated 5 days at room temperature. Then 50 seeds of acid-delinted cotton (var. Coker 100) were planted in each container. Each treatment was randomized and replicated at least 3 times. Notes were made periodically for 14 days on the number of seedlings that died as a result of postemergence damping-off. On the fourteenth day the total number of plants killed was recorded. The basidiospore isolates were given a virulence rating based on per cent cotton seedlings killed as a result of both pre- and postemergence damping-off. Three basidiospore isolates (21, 23, and 50) of differing virulence were selected for further study.

Inoculum standardization.—For accurate determination of the changes in virulence that occur following culture of the selected isolates, inoculum standardization was necessary. Differences in weight were obtained following culture of the selected isolates at different temperatures on different culture media. A dry-weight method of inoculum standardization was devised. The mycelial mats obtained following culture of each isolate for 7 days on basal medium at 20°C and 30°C were blended separately for 20 seconds in a Waring Blendor with 25 ml of water per mat. Five-ml aliquots of the resulting mycelial suspension were poured onto filter paper of known weight in a Buchner funnel attached to a suction-trap flask. Water was allowed to drain from the suspension by suction, and the filter paper containing the mycelial fragments was allowed to airdry for 24 hours and then weighed on an analytical balance. Calculations were made to determine the weight of the dried mycelium that was in each 5-ml aliquot of the different mycelial suspensions. Three such determinations were made for each different suspension of each isolate in each treatment, and the average weight obtained was used for further standardization. The average dry weights of mycelium obtained from the 5-ml suspension differed among isolates and among treatments within isolates. Since the standardization was based on weight per volume, it was desirable to use mycelial suspensions of isolates having the same mycelial weight. The volume of each suspension of each isolate had to be adjusted. The average weight of dried mycelium obtained from a 5-ml suspension made from mycelial mats of isolate 23 cultured 7 days at 20°C on the vitamin-free basal medium was found to be 25 mg, enough to cause about 97% damping-off and seedling decay of 20 cotton seedlings in a pint of steamed soil. A volume of a mycelial suspension that contained 25 mg of dried mycelium was called the X weight-volume concentration. For

Fig. 1.—Virulence of basidiospore isolate 23 to cotton plants after culture on biotin-amended media maintained at 20°C and 30°C, compared with its virulence after culture on basal medium maintained at the same temperatures. Front row, 20° series; back row, 30° series. Far right, uninfested check. Far left, basal infested check.

further experiments, all subsequent mycelial suspensions of each isolate were adjusted to X concentration or multiples thereof.

Experimental results.—*Virulence of 3 basidiospore isolates as affected by different culture media and temperatures.*—Basidiospore isolates 21, 23, and 50 were cultured 14 days on vitamin-free and on 4 vitamin-amended basal media in 250-ml Erlenmeyer flasks maintained at 20°C and 30°C. Each treatment was replicated 6 times. After a 14-day culture period, the mycelium from each treatment was standardized by the dry-weight method previously described. Standardized mycelial suspensions of each treatment among isolates and within isolates were mixed in pints of steamed soil in the X, 2X, and 3X weight-volume concentrations and placed in waxed pint containers and allowed to remain 5 days at room temperature. Fifty ml of water were placed in each container daily for 5 days, and on the sixth day 20 seeds of cotton were planted in each pint of infested and uninfested soil. The containers were then completely randomized on a greenhouse bench. On the 14th day the number of surviving plants was recorded. This experiment was repeated twice and the data from all tests statistically analyzed.

Basidiospore isolates 21 and 50 were not virulent after culture on the vitamin-free basal medium at the concentration of inoculum used. The experiment was repeated for these isolates with 8X, 10X, and 20X weight-volume concentrations of inoculum. At these concentrations, virulence of the two isolates was proportional to the concentration of inoculum. Changes in virulence of the two isolates were then investigated following culture on the vitamin-free basal medium and the vitamin-amended basal media in the manner previously described.

Basidiospore isolate 23 was significantly less virulent when the X, 2X, and 3X weight-volume concentration of inoculum was used following culture of this isolate on all vitamin-amended media at 30°C, but not at 20°C (Fig. 1). Culture of the same isolate on the biotin plus thiamine medium (hereinafter called BT medium) resulted in the greatest decrease in virulence, followed in order by culture on inositol, biotin, and thiamine amended media. Virulence of isolate 23 at all weight-volume concentrations was also significantly less following culture on BT medium at 30°C than following culture on vitamin-free basal medium under the same conditions (Table 1). An increase in the weight-volume concentration of mycelium was positively correlated with an increase in virulence. When the experiment was repeated, basidiospore isolate 23 was significantly less virulent following culture on all vitamin-amended media maintained at 30°C and significantly less virulent following culture on BT medium at 20°C. In this second experiment, only the X weight-volume concentration was used. The same treatments, however, in the order named, caused significantly more postemergence damping-off. The amount of damping-off in each treatment was proportional to an increase of the inoculum concentration.

Comparison of 2 single-basidiospore isolates with

Table 1.—Virulence of basidiospore isolate 23 following culture at 30°C on vitamin-free basal medium and basal medium amended with biotin plus thiamin. Virulence measured by number of plants remaining 14 days after planting cotton seeds in steamed soil infested with 3 weight-volume concentrations of standardized mycelium. Treatments replicated 3 times

	Weight-volume concentration of mycelial suspension					
	Basal			BT[a]		
	X	2X	3X	X	2X	3X
	3	1	0	18	12	2
	0	0	0	18	13	7
	0	0	0	17	10	6
Totals	3	1	0	53	35	16

Vitamin totals	Concentration totals
Basal—4	X—56
BT—104	2X—36
	3X—16

LSD for totals at 5% level: 8

[a] Basal medium amended with biotin plus thiamine.

the parent basidiospore isolate 23 in cultural characteristics, mycelial weight, and effect of culture substrates on virulence.—Two single-basidiospore isolates were obtained from a reisolation of basidiospore isolate 23 from diseased cotton seedlings in the manner previously described. Cultural characteristics were compared on PDA, basal agar, and yeast extract agar.

The isolates were cultured on different vitamin-amended basal media and on the vitamin-free basal medium at 20°C and 30°C. Their growth on these media and their virulence following culture on these media were compared to the parent isolate, using the methods previously described.

When the 2 single-basidiospore progenies of isolate 23 (hereinafter called 23A and 23B) were compared with each other and with the parent isolate in mycelial weight and cultural characteristics on three different media (PDA, basal agar, and yeast extract agar), differences were noted. The colony diameters of each isolate appeared to be the same after 7 days (visual observation). On basal agar, all isolates differed culturally. Sectoring was evident in the culture containing basidiospore isolate 23A. The greatest similarities in cultural characteristics of the isolates were observed on PDA and on yeast extract agar cultures (Fig. 2).

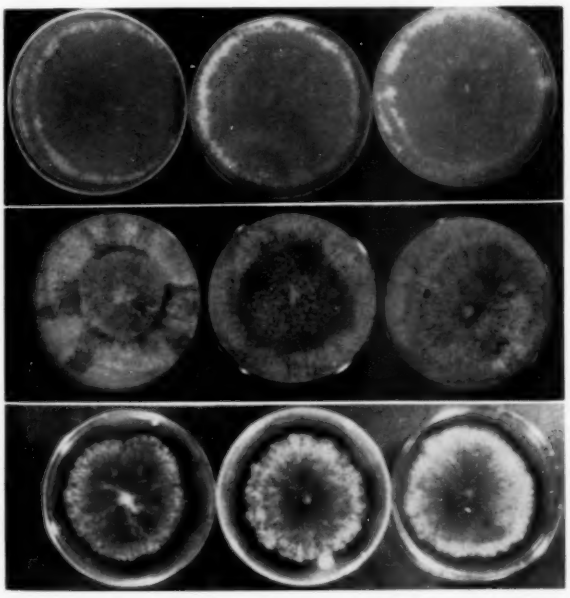

Fig. 2.—Seven-day-old cultures of basidiospore isolates 23, 23A, and 23B on 3 different substrates. Left to right: 23B, 23A, and 23. Top to bottom: PDA, basal agar, and yeast extract agar.

Basidiospore isolates 23, 23A, and 23B differed significantly from each other in mycelial weight after culture upon either the vitamin-free basal medium or the vitamin-amended basal media. The mycelial weight of isolate 23A was greater than that of the other two, and that of 23B was greater than that of 23. No significant differences in mycelial weight occurred as a result of culture of these isolates at either 20°C or 30°C, although at both temperatures the mycelial weights of isolates cultured on vitamin-amended media differed significantly from those cultured on the basal vitamin-free medium.

There were no significant differences in the virulence of basidiospore isolates 23, 23A, and 23B following culture on vitamin-amended basal media. All isolates were significantly less virulent following culture on these same media than following culture on the vitamin-free basal medium (Table 2). Virulence of the same isolates, as measured by postemergence damping-off, following culture on vitamin-amended media was not significantly different, although culture media and temperature of culture on those media significantly affected postemergence damping-off. Culture on inositol medium resulted in the greatest amount of postemergence damping-off, whereas culture on thiamine medium resulted in the least amount.

Discussion.—Changes in pathogenicity and virulence that occur in fungi as a result of culture in various environments have not been thoroughly investigated. It is necessary to determine whether the changes that occur are due to selection of certain biotypes from an original mixture of biotypes or from mutations, hetero-

Table 2.—Virulence of 2 basidiospore isolates on cotton seedlings as compared to that of the parent isolate following culture on vitamin-free basal and vitamin-amended basal media at 20°C and 30°C. Data are numbers of plants remaining 14 days after planting 20 cotton seeds in steamed soil infested with standardized mycelial suspensions of each isolate. Each treatment replicated 4 times

Media	Isolates		
	23	23A	23B
Basal	3[a]	24	1
Biotin	7	1	6
BT[b]	23	15	1
Thiamine	30	4	42
Inositol	4	7	3
Isolate totals	67[c]	51	53

[a] LSD for media totals at 5% level: 9

[b] Basal medium amended with biotin plus thiamine.

[c] Isolate totals not significantly different.

karyoses, or nuclear disassociations in either the culture environment or in the immediate environment or both. If the fungus is one that has nuclei all of one kind, it is necessary to know whether the changes that occur in virulence following increase in the culture environment are due to mutations, followed by selection of the mutant or nonmutant, or whether the changes are due to differences in the genetic potential of the fungus in a particular environment. Changes in virulence of single-mononucleate basidiospore isolates of *Pellicularia filamentosa* following culture on vitamin-free basal medium and on vitamin-amended basal media maintained at two temperatures were investigated. This was followed by an investigation of changes in virulence in 2 of the single-mononucleate progenies of one of the isolates used.

One single-basidiospore isolate (Isolate 23) was significantly less virulent following culture at 20°C and 30°C on vitamin-amended basal media than following culture on a vitamin-free basal medium at the same temperatures. Culture of the isolate on vitamin-free basal medium amended with biotin and thiamine had the greatest effect. The culture temperature was not significant as a factor affecting a decrease in virulence when all experiments were considered. However, in separate experiments culture of the isolate at 20°C was found to be significant and in another separate experiment culture of the isolate at 30°C was found to be significant. The mechanism by which vitamins in the culture media cause a reduction in virulence of this fungus is not clearly understood. In preliminary work, 3 of the 4 vitamins used inhibited growth of Isolate 23 as well as other isolates in culture. It is possible that the vitamins used caused the inhibition of enzymatic activity and toxin formation of this isolate, and that it is correlated with inhibited growth of the isolate in the culture environment. The inhibition of toxin formation and enzyme activity probably persists when the isolate is transferred to the inoculation environment. This inhibition persists longer in isolates cultured on BT medium. However there is a diminution of inhibition as evidenced by more postemergence damping-off of cotton seedlings growing in soil infested with this isolate.

To determine whether the lessened virulence of basidiospore isolate 23 was a result of a genetic change, or whether it was a change induced by the different vitamin-amended substrates on the rates and kinds of processes, 2 single-basidiospore isolates, progenies of basidiospore isolate 23, were compared with the parent. If the progenies were like the parent in morphology and physiology, one could assume that a genetic change did not occur in the parent. If, however, the parent differed from the progenies in morphology and physiology, it would be assumed that a genetic change occurred.

When the progeny isolates were compared with the parent isolate, no significant differences occurred in total virulence (i.e. virulence due to pre- and postemergence damping-off), and total virulence of all isolates (parent and progeny) was significantly less after culture on all basal vitamin-amended media. Although no significant differences occurred in virulence between isolates, they differed significantly in the treatment that contributed to a decrease in virulence. The progeny isolates differed significantly from the parent in the culture temperature that caused a decrease in virulence as well as in total virulence. Postemergence damping-off between parent and progeny was not different, but the treatments that caused postemergence damping-off were different.

It is probable that the differences between parent and progeny may have been due to mutations, differences in substrate, and chance variation that could not be controlled, or to a combination of all three. Mutations may have occurred in some nuclei of both the progeny and parent isolates, but were not sufficient to cause a selection of either the non-mutant or mutant nuclei. However, nuclear ratios may have changed following mutation of a nucleus, which would result in differences in rates and kinds of processes of all isolates. Such differences evidently were not sufficient to cause a change in virulence between isolates. Parent and progeny were less virulent following culture on vitamin-amended substrates, probably because of changes in physiology induced by substrate. It is significant, however, that these changes were reversible, since, after culture on basal medium, both parent and progeny retained their original degree of virulence. —Department of Botany and Plant Pathology, The Ohio State University, Columbus 10.

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SOIL INDEXING FOR PEA ROOT ROT AND THE EFFECT OF ROOT ROT ON YIELD

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SUMMARY

In random samples of soil from fields in which peas were to be planted, peas known to be susceptible to root rot caused by *Aphanomyces euteiches* and *Fusarium solani* f. *pisi* were grown. The plants were removed at full bloom and the amount of root rot recorded. The amount of root rot was recorded later that developed in peas planted in the fields from which the soil samples were taken. The tests comprised 76 fields in 1955, 66 in 1956, and 60 in 1957. The amount of root rot that developed in peas planted in soils in the greenhouse showed highly significant correlation with the amount of root rot that developed in the fields from which these soils came. It was also found that as the degree of root rot increased the vine and pod yield decreased, and early varieties, such as Alaska, are probably more tolerant to root rot than the late varieties.

Root rot of canning peas is one of the major pathological factors in pea production. Of the various fungi causing root rot of peas in the Midwest, *Aphanomyces euteiches* Drechs. and *Fusarium solani* (Mart.) App. & Wr. f. *pisi* (F. R. Jones) Snyd. & Hans. are the most important. Since the present commercial varieties offer no sources of resistance to these fungi, a method of land selection would be valuable for avoiding severe crop losses from root rots.

Methods of sampling soils to determine pathogen content prior to growing a crop have been found useful in the past. Other workers have used seedlings as a means of determining how badly soils were infested with fungi (1, 3, 4). Johnson (2) recently reported a similar method of using peas to demonstrate the degree of infestation of soils with common root rot and Fusarium root rot.

The purposes of the present study were: to determine the efficiency of Johnson's method for testing and predicting the degree of soil infestation with root rot in the field from greenhouse determinations; and to determine the effect on yield of various degrees of root rot infection in peas.

Materials and methods.—The method developed by Johnson (2) for determining the severity of root rot of peas in soil was modified when necessary to increase the efficacy of the test.

From each commercial field to be tested for the occurrence of the root rot organisms 10 random soil samples were collected in the fall of each year preceding a spring planting. The samples from each field were bulked, constituting 2 gal. of soil from each of 76 fields in 1955, 66 fields in 1956, and 60 fields in 1957. Samples were placed in 2-gal. ice cream cartons in 1955 and in polyethylene bags for shipping and storage in 1956 and 1957.

Fig. 1.—The effect of various degrees of root rot severity on the growth of peas of the varieties New Line Early Perfection (left of each pot) and Wasatch (right side) as indicated by soil sample SV 4S (severely affected), SV10M (moderately affected), and LM4X (unaffected). Pot on the left of each soil sample was autoclaved to eliminate the root rotting organisms.

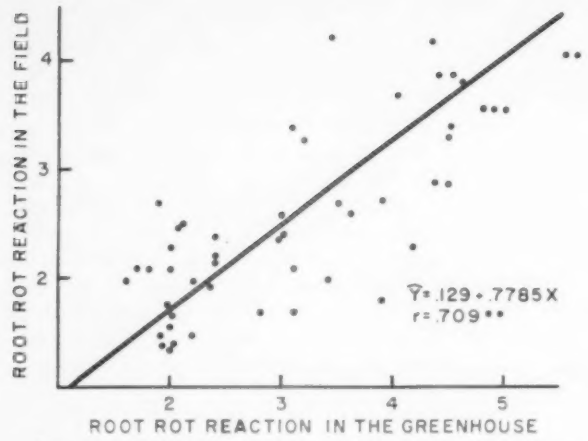

Fig. 2.—Correlation between pea root rot development in greenhouse tests and root rot development in 49 fields in 1955.

Each soil sample was thoroughly screened and mixed before being placed in four 5-in. clay pots and planted to 2 indicator varieties, Wasatch and New Line Early Perfection. Six seeds of each indicator variety were planted in each pot of soil, with the 2 varieties separated by a paper card that divided the pot in half. One pot of soil of each sample was autoclaved, planted, and maintained as a check on root rot development.

After emergence, the seedlings were thinned to 4 plants per variety per pot. Each soil type was watered as a separate unit in order that a more uniform soil moisture could be maintained between the various soil types. Before wilting point was reached, enough water was applied to moisten all the soil thoroughly.

The plants were harvested at the early bloom of Wasatch—about 30–40 days after planting when grown at a night temperature of about 65°F and a day temperature of 70–75°F. The amount of root rot was numerically rated as follows: 1–2, clean roots; 2–3, light infection; 3–4, moderate infection; 4–5, severe rotting; and 6, death of the infected plant. Fig. 1 illustrates some of the differences in root rot development when peas were grown in the various soil samples.

The following summer, after peas growing in the previously sampled fields had reached the full-bloom stage of maturity, 5–20 randomly selected samples, each sample containing 32 pea plants were taken from each field. Each plant was examined and rated as to the amount of root rot, and the samples were averaged to indicate actual root rot in each commercial field. No attempt was made in this study to determine specifically the organisms causing the root rot on each plant. However, results of previous studies indicated that *A. euteiches* and *F. solani* f. *pisi* were the most prevalent root rotting organisms in infested fields.

Experimental results.—As shown in Fig. 2, 3, and 4, soil taken from 154 fields was evaluated in the greenhouse for the severity of root rot. The predicted root rot reaction was then compared with the actual severity of root rot that developed on peas grown in the sampled fields.

The predictions of root development from greenhouse determinations proved to be very accurate in 1955 and 1957, as illustrated in Fig. 2 and 4. The regression coefficients for 1955 and 1957 were 0.709 and 0.617, respectively. In 1956, the regression coefficient was 0.458 (Fig. 3). Statistically, this correlation is highly significant; however, the accuracy for this year is not as high as expected.

In 1956, the largest deviation from the regression line occurred with the 22 soil samples from the fields from the area near Spring Valley, Minnesota, whereas the samples from the other areas used in the study were, for the most part, concentrated around the regression line.

In 1955 the average rating of root rot was lower in the field than in the greenhouse, in 1956 the reverse was true, and in 1957 the greenhouse and field ratings were about equal.

As indicated in the results, Johnson's method of determining the severity of pea root rot (2) is accur-

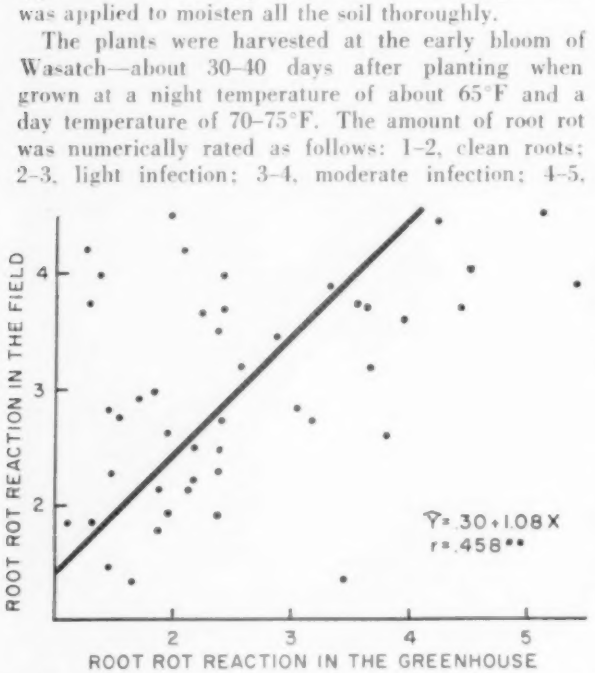

Fig. 3.—Correlation between pea root rot development in greenhouse tests and root rot development in 45 fields in 1956.

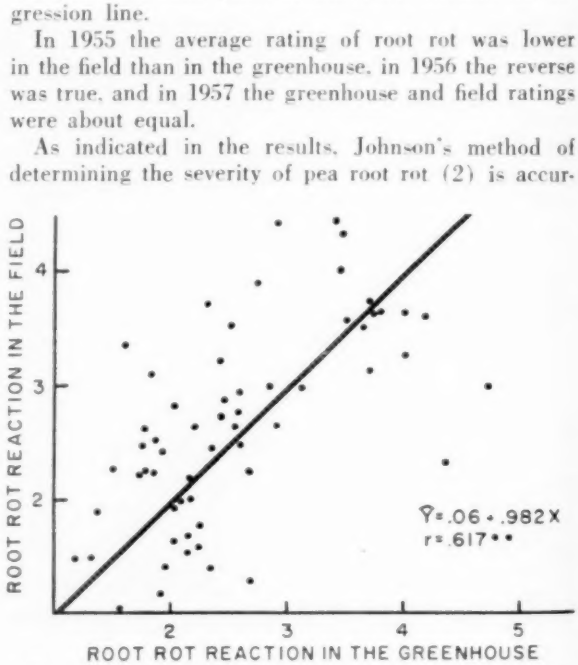

Fig. 4.—Correlation between pea root rot development in greenhouse tests and root rot development in 60 fields in 1957.

Table 1.—A comparison of average root rot ratings of soil samples taken from fields that had various degrees of pea yield reduction in 1954 and 1955

Year	Average root-rot rating[a] from greenhouse from fields with			
	Severely reduced yields	Moderately reduced yields	Good yield	No previous peas
1954	4.1	4.4	3.4	1.4
1955	4.1	4.0	3.2	1.5

[a] 1–2, clean roots; 3, moderately rotted; 4–5, severely rotted.

ate. Fields free of root rot infestation and severely infested fields are easily determined. In moderately infested fields, environmental conditions during the growing season may be the most influential factor, making accurate prediction virtually impossible. In any case, the canner can develop a realistic program of land selection.

Many fields planted to peas in 1954 and 1955 were abandoned by canning companies and farmers because of poor stands or yields considerably lower than expected. Soil samples were taken and the severity of root rot determined in the greenhouse to find whether root rot was a major factor. In 15 of the 41 fields sampled, yields had been severely reduced, in 15, yields were moderately low, in 6 yields were good, and in the other 5, yields were good though the fields had never grown a commercial crop of peas.

Of the 15 soil samples from fields that had severely reduced yields, all but one were severely infested with root rot, all of the soil samples from fields of moderately reduced yields were infested with root rot, 4 of 6 of the soil samples from good-yielding fields were moderately infested, and all 5 soil samples from fields with no previous history of cropping to peas produced plants with rot-free roots. Table 1 shows the average root rot indexes of plants grown in soil from fields with the 4 different cropping histories. The results indicate that a significant amount of the reduction in yield in many of the commercial fields was due to root rot.

Effect of root rot severity on the yield of pea varieties.—As indicated in the preceding results, a reliable method has been developed for predicting root rot development on peas planted in sampled fields. Although it appears that the degree of infestation of soils can be determined, the relative effect of root rot on yield is unknown.

Therefore an attempt was made to determine the effect of relative degrees of root rot on the yield of various varieties of canning peas. The degree of root rot development was determined in 159 fields of canning peas, and rated: 1–2, little or no root rot; 2–3, light infection; 3–4, moderate infection; and 4–5, severe infection.

Table 2 presents the average of 3 years' data from 9 commercial pea varieties having various degrees of root rot: 1–2, a clean field; 2–3, 9.6% reduction in yield; 3–4, 22.3% reduction; and 4–5, 33.1% reduction.

It is also evident that in some varieties root rot was severe but there was little or no reduction in yield. It is known that many factors affect yield, such as previous cultural practices, environmental conditions, rate and time of seeding, plant stand, pea variety, time of infection, stage of plant development and other diseases. In spite of these factors as well as the total variability in growing conditions in the three years, yield was reduced as the average degree of root rot increased.

Effect of root rot on vine and pod weight.—In previous studies, it was found that the yield of early varieties such as Alaska was not reduced as severely as late varieties when they were planted in severely infested fields. Therefore in 1956 a study was made to determine the effect of the severity of root rot on the growth of pea vines and pods. Four 50-plant samples having various degrees of root rot were harvested at about the preharvest stage of plant development. These samples were obtained from commercial pea fields in Minnesota and Wisconsin that were heterogenous with respect to root rot severity. All plants were pulled and the degree of root rot determined before placing each in one of the various categories of root rot. Four

Table 2.—The effect of various degrees of root rotting on the yield (lb./A.) of nine varieties of canning peas for the three years 1955 to 1957

Pea variety	Degree of root rot[a]							
	1–2		2–3		3–4		4–5	
	No. fields	Yield	No. fields	Yield	No. fields	Yield	No. fields	Yield
Alaska	4	2091	10	1689	14	1534	1	1301
Allsweets	4	2976	11	2110	7	2150		
New Line E. Perf.	5	2824	6	3434	9	2095		
E. Perf. 109	4	2835	3	3730	3	3546		
Canner King	5	2762	8	3008	8	2849	2	2796
Laurel	4	3497	8	2677	2	1912		
E. Harvest	2	3527	7	2696	3	1738		
Superior	3	2946	5	3266	6	2936	2	1820
Cascade	2	3444	3	2803	5	2917	2	1573
Av. yield		2919		2639		2269		1954
Difference from clean fields				−280		−650		−965
% of 1-2 rating				90.4%		77.7%		66.9%

[a] 1–2, light to no root rot; 2–3, light to moderate root rot; 3–4, moderate to heavy root rot, 4–5, severe root rot.

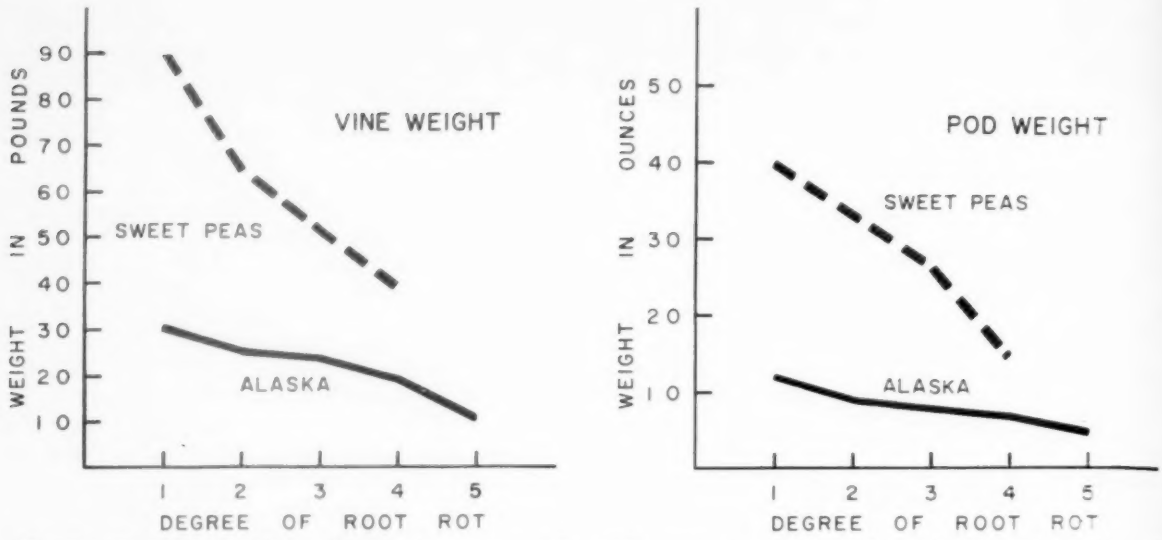

Fig. 5.—The effect of different degrees of root rot severity on the mean vine weight (left) and pod weight (right) of Alaska and high-sugar varieties of peas in 1956.

fields of Alaska peas and 3 fields of high sugar varieties were sampled in this study. Of the 3 fields of the high sugar varieties examined, 1 was planted to the variety Canner King and the other 2 to variety Cascade.

Fig. 5 presents the effect of root rot severity on pod weight and vine weight from 50 plant samples of Alaska and sweet peas.

It is evident that, with increase in root rot, vine weight and pod yield declined more rapidly in Canner King and Cascade than in Alaska. In Alaska peas, pod weight was decreased 54% and vine weight 35% as the severity of root rot increased (from 1 to a 4); whereas, with Canner King and Cascade yield of pods declined 63% and vines 65% as root rot rating increased from 1 to a 4. The results indicate that an increase in root rot severity directly affects plant growth, and that Alaska peas are probably more tolerant to root rot than the late varieties. However, if infection occurs early in the stage of plant development, yield is severely affected.—Department of Plant Pathology and Botany, University of Minnesota, Institute of Agriculture, St. Paul 1.

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STUDIES ON THE STRIPE MOSAIC OF BARLEY, INCLUDING EFFECTS OF TEMPERATURE AND AGE OF HOST ON DISEASE DEVELOPMENT AND SEED INFECTION

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SUMMARY

The barley stripe mosaic virus produced suggestive symptoms in a few species outside the Gramineae, but the virus could not be recovered. In susceptible wheat, barley, and corn the disease severity increased with increasing soil and air temperatures. The age of the plant at inoculation and also the postinoculation air temperature influenced the reaction of and seed-transmission in 4 tolerant barley varieties. In the field these varieties became infected at all stages of growth, but were most affected when inoculated before the boot stage. Also in the field, these varieties gave less than 15% seed-transmission, while the susceptible variety gave up to 75%. In the greenhouse the tolerant varieties showed little or no symptoms at 16°C, but were severely affected at 24° and 28°. Seed-transmission was maximum at 20° and 24°.

The occurrence of stripe mosaic (false stripe, as it was called earlier) in barley is reported (10) to have been noted in the United States as early as 1913. Failure to associate a pathogenic agency with the

disease led earlier workers (3) to describe the malady as a physiological disorder. In 1949, Wadsworth (22) reported a barley mosaic from Oklahoma and showed that it could be mechanically transmitted. This was later (12) shown to be stripe mosaic. In 1951, McKinney (10) reported that "false stripe" symptoms were caused by a seed-borne virus and gave a brief account (11) of the disease and the associated virus. In the same year, Hagborg (7) also found evidence that the disease was caused by a virus. Further reports indicated that the disease was becoming a serious threat to crops in several sections of the United States (4, 6, 21) and Canada (7). A similar disease has been reported in Japan (20), England (9), and Germany (19).

This paper reports studies dealing with host range and with the influence of temperature and age of plant on symptoms and seed-transmission in susceptible and resistant host plants.

Host range.—Numerous species in the Gramineae have been reported as susceptible to the stripe mosaic virus. The virus is readily transmissible to barley, wheat, rye, corn, sorghum, and a number of grasses (5, 10, 12, 13, 17, 18, 21). Rice (11) and oats (12) have given local lesions, although certain strains become systemic in oats (17). Three species outside the grass family have been reported as local-lesion hosts: *Nicotiana tabacum* L. var. Samsun (11, 12), *Chenopodium album* L. (13), and *C. amaranticolor* Coste & Reyn. (9). Holling's work on *C. amaranticolor* (9) was not available until after the present study was completed.

Since no local-lesion host suitable for assay of this virus was known at the start of this work, plants distributed among 34 genera and 12 families were tested for their reactions. Infected seeds of Manchuria barley, C.I. 2330 (accession number of the Cereal Crops Research Branch, Crops Research Division, U.S.D.A.), supplied by R. G. Timian, were used as source of the stripe mosaic virus. The virus, designated Manchuria isolate (21), was maintained in plants of Oderbrucker barley (C.I. 4666). The inoculum was juice extract from these plants, prepared by triturating frozen tissues in a 0.1*M* phosphate buffer of pH 6.8. Plants were inoculated by rubbing leaves with a cheesecloth pad that had been dipped in infectious extract. Carborundum was added to the inoculum or dusted on the leaves before inoculation. With grasses, both sides of leaves were inoculated. The inoculated leaves were immediately rinsed with tap water. Following inoculation, plants were kept at about 20°C air temperature. To test for the virus in a given host, subinoculations were made to Oderbrucker seedlings.

Plants that were readily infected, and from which the virus could be recovered, were Henry wheat (*Triticum aestivum* L.), Wis. 749 × 927 sweet corn, yellow dent field corn, Inbred A19 albino corn (*Zea mays* L.), Adams rye (*Secale cereale* L.), *Panicum virgatum* L., and bent grass (*Agrostis palustris* Huds.). With Branch oats (*Avena sativa* L.) and brome grass (*Bromus inermis* Leyss.), systemic infection did not occur although brown necrotic lesions were occasionally produced on the inoculated leaves. Plants that developed suggestive symptoms of virus infection but failed to yield the virus were Canada blue grass (*Poa compressa* L.), intermediate wheat grass (*Agropyron intermedium* (Host.) Beauv.), red fescue (*Festuca rubra* L.), lambsquarters (*Chenopodium album* L.), strawberry blite (*C. capitatum* (L.) Asch.), pigweed (*Amaranthus retroflexus* L.), and buckwheat (*Fagopyrum esculentum* Gaertn.). On these hosts, indistinct chlorotic or distinct necrotic lesions were produced on inoculated leaves. Local lesions produced on the leaves of *Chenopodium album* could be reproduced by subinoculation to the same host, but not to barley. In addition, the lesions were not distinct enough to be usable in quantitative tests.

Plants that were apparently immune from the Manchuria isolate at an air temperature range of 20–28° were rice (*Oryza sativa* L.), Piper sudan grass (*Sorghum vulgare* var. *sudanense* (Piper) Hitchc.), Leoti sorghum (*S. vulgare* Pers.), timothy (*Phleum pratense* L.), wild oats (*Avena fatua* L.), yellow fox-tail (*Setaria lutescens* (Wiegel) Hubb), orchard grass (*Dactylis glomerata* L.), Bountiful, Idaho Refugee, Michelite, Navy Pinto, and Red Kidney bean (*Phaseolus vulgaris* L.), Fordhook Lima bean (*P. limensis* Macf.), mung bean (*P. aureus* Roxb.), soybean (*Glycine max* Merr.), black cowpea (*Vigna sinensis* (L.) Endl.), guar (*Cyamopsis tetragonoloba* (L.) Taubert), Perfected Wales pea (*Pisum sativum* L.), Samsun tobacco (*Nicotiana tabacum* L.), World Beater and Yolo Wonder pepper (*Capsicum annuum* L.), spinach (*Spinacia oleracea* L.), sugar beet (*Beta vulgaris* L.), butter and eggs (*Linaria vulgaris* Hill.), wild bergamot (*Monarda fistulosa* L.), castor bean (*Ricinus communis* L.), onion (*Allium cepa* L.), sunflower (*Helianthus annuus* L.), okra (*Hibiscus esculentus* L.), and knot-weed (*Polygonum lapathifolium* L. and *P. pennsylvanicum* L.). Failure to infect rice, tobacco, and sorghum is at variance with earlier reports (11, 18), but may have been due to strain differences.

Sap extracts from infected lambsquarters leaves usually produced symptoms on the same host, but failed to give any reaction on susceptible barley. As a possible explanation for the failure, the presence of some inhibitory substance in the leaves of those plants was explored.

Sap extracts from uninoculated lambsquarters plants were obtained by grinding leaves with 0.1*M* phosphate buffer. Aliquots were mixed, in equal proportions, with inoculum obtained from infected barley plants. Lambsquarters and barley plants were then inoculated with the adulterated inoculum at 28° air temperature. The experiments were repeated several times. Lambsquarters plants inoculated with the adulterated inoculum developed local lesions in 5–6 days, whereas plants rubbed with juice from healthy lambsquarters showed no reaction. Barley plants inoculated with the adulterated inoculum consistently failed to show any symptoms. Unadulterated inoculum from virus-infected barley invariably produced

Table 1.—Effect of air temperature on symptom severity of barley stripe mosaic

Temperature (°C)	Disease indices[a] at given days after inoculation					
	Barley		Wheat		Corn	
	10	20	10	20	10	20
16	9	24	1	35	0	10
20	28	45	34	49	13	26
24	41	63	50	72	24	47
28	62	72	72	83	56	74

[a] 0 = no symptoms in any plants; 100 = all plants killed. Individual plants rated according to severity of symptoms. Values shown are averages of 2 experiments.

stripe symptoms on barley and local lesions on lambsquarters. An inhibitory substance was apparently present in the lambsquarters extracts that prevented the virus from being transferred back to the barley host, but did not prevent its transfer from lambsquarters to lambsquarters.

Effect of temperature on symptom expression in susceptible plants.—*Air-temperature relations.*—McKinney (13) did considerable work on the effects of temperature and light on symptom expression in tests of a virus infected seed lot of Glacier barley, a variety in which mild symptom producing virus strains predominated. He reported that temperatures of 75–100°F with adequate light favored the expression of the chlorotic markings in the first 3 leaves. Hampton et al (8) found that temperatures from 70 to 85°F and light intensities near 10,000 ft-c were optimum for development of seedling symptoms in test of infected seed lots.

In the present work the effects of temperature on the disease in mechanically inoculated plants were studied. This work was done during the winter time in the greenhouse so that light was frequently less than optimum.

Barley (Oderbrucker), wheat (Henry, C.I. 12265), and sweet corn (Wisconsin Hybrid 749×724) plants were grown in sand culture (14) at about 20° air temperature. Plants were inoculated at the 2–3-leaf stage, as described above. Following inoculation, plants were divided among greenhouses held at 16°, 20°, 24°, and 28° air temperatures. Each infected plant was rated for symptom severity and placed in one of 5 classes: 0, no symptoms; 1, slight symptoms—small gray to brown discontinuous stripes on the inoculated leaves, mild systemic mottle; 2, moderate symptoms—conspicuous bleaching of leaf tips; 3, severe symptoms—necrosis of leaf apices; 4, very severe symptoms—top necrosis and death of plant. In calculations of the disease index, the number of plants in each class was multiplied by the class number in which those plants were placed. The sum of the products was multiplied by 100 and the result divided by four times the total number of plants observed.

At 16° the incubation period was 9–13 days on wheat and barley, and 14–19 days on corn. At 28°, symptoms appeared in 4–6 days on wheat and barley, and 7–8 days on corn. At all temperatures, first symptoms consisted of gray to brown lesions on inoculated leaves. Systemic infection gave mild to severe mosaic mottle at 16° and 20°; and severe chlorosis, and often necrosis of infected leaves at 24° and 28°.

Symptom severity on all crops increased with increasing temperatures (Table 1). Some of the infected plants at 28° showed systemic necrosis and were eventually killed. Wheat showed the most severe symptoms at all temperatures. Symptom development was slower on corn than on wheat or barley at the three lower temperatures. Occasionally, when top necrosis of corn plants occurred at 28°, the newly developing leaves pierced through the enclosing leaf sheaths and showed gnarled, anomalous growth (Fig. 1).

Surviving wheat and barley plants held at 28° and 24° showed apparent recovery about 25–30 days after inoculation. This was most marked on the young leaves. About 30 days after inoculation, plants at 16° and 20° developed severe symptoms on young leaves. Although the readings in Table 1 do not indicate this, the symptom expression on plants grown at low temperatures eventually became as severe as that on surviving plants at high temperatures. On corn, symptoms were always most severe on plants grown at 28°.

These results suggested that, within the range of temperatures under study, corn was less susceptible to the virus isolate than were wheat and barley. With susceptible wheat and barley the initial symptom development was most severe at 28°C, but symptom expression on the surviving plants eventually was as pronounced at low temperatures as at high temperatures.

Soil-temperature relations.—Since differences in air

Fig. 1.—Anomalous growth of sweet corn infected with barley stripe mosaic virus and grown at 28°C.

temperature were observed to influence the symptoms of the stripe mosaic on wheat, barley, and corn, further experiments were carried out to see if reactions on these hosts would be similar at different soil temperatures. The range of soil temperature, methods of growing plants, and inoculations were essentially as described above. Air temperature was maintained at 22°.

The effect of soil temperature on symptoms was less marked than that of air temperature. Systemic symptoms appeared on wheat and barley 8–12 days after inoculation, and on corn in 9–16 days. Wheat appeared to be most susceptible at all soil temperatures. Symptoms on all hosts were more pronounced and severe at 28° and 24° than at 20° and 16°.

Effect of air temperature on tolerant barleys.—In view of recent reports indicating the marked influence of air temperature on expression of resistance of some crops to certain viruses (2, 14, 15, 16), it was of interest to see if a similar situation existed with barley varieties that have been shown by Timian and Sisler (21) to have some tolerance to the stripe mosaic virus.

In these experiments, barley varieties C.I. 5020, C.I. 3212 (Modjo), C.I. 3212-1, and C.I. 4219 were considered. In the following presentation, these varieties are referred to by the number only. Seeds of these varieties were obtained from R. G. Timian and D. J. Ward, of the U.S.D.A. For comparison, Oderbrucker was included in all experiments.

Air temperature had a marked influence on the growth of both inoculated and uninoculated plants. At 16°, plants developed profuse tillers and were highly succulent. Growth of inoculated and uninoculated plants was maximum at 20° or 24°. Plants of all varieties grown at 28° were slightly stunted and showed few tillers.

The incubation period of stripe mosaic decreased with an increase in temperature. Systemic infection in 5020 and 3212 at 16° was evident within 25–30 days of inoculation. Varieties 3212-1 and 4219 failed to show systemic symptoms at this temperature. At 20°, symptoms on 5020 and 3212 developed in 9–10 days, while 3212-1 and 4219 showed symptoms 15–16 days after inoculation, but effects were not as severe as in the 2 former lines. At 24°, reactions were intermediate between those at 20° and 28°. At 28°, 5020 and 3212 developed symptoms in 4–6 days and occasionally showed systemic necrosis of leaves, whereas 3212-1 and 4219 showed infection in 6–8 days, but without necrosis.

Symptom severity in the tolerant varieties increased with increasing temperatures, but even at 28° was not as severe as in susceptible Oderbrucker. Symptom development on the tolerant varieties also was slower than on Oderbrucker.

Oderbrucker and 3212-1 are compared in symptom development and severity in Table 2. Symptoms in this experiment on Oderbrucker were not as severe as those observed in experiments discussed above. The variety 3212-1 was highly resistant at low temperatures but moderately susceptible at 24° and 28°.

When infected plants were moved 12 days after inoculation from 16°, 20°, 24°, and 28° to 28°, 24°, 20°, and 16°, respectively, systemic symptoms on all varieties developed in accordance with the final temperature conditions. Varieties 3212-1 and 4219, which showed no systemic symptoms at 16°, developed mild systemic symptoms 6–7 days after they were transferred to 28°. In all varieties the disease syndrome was similar at this temperature to that on plants grown at 28° continuously. When plants grown at 28° were moved to 16°, most of the infected leaves collapsed within 7–8 days of transfer. This reaction was most rapid and conspicuous in Oderbrucker. Newly produced leaves were almost symptomless except in Oberbrucker and 5020, where mild mosaic mottle developed on the young leaves. At intermediate temperatures reactions were intermediate. These results suggested that, although the tolerant lines are susceptible to the virus, virus-multiplication at low temperatures is probably so restricted that symptoms do not develop.

Effect of age of plant on symptom expression in barley.—Field trials with different varieties of barley have shown that differences in yield reduction due to the virus may depend on the initial infection of the seed used (4, 21), on the susceptibility of the varieties to the stripe mosaic virus during growth, and on the growth stage at which infection occurs (5).

Eslick and Afanasiev (5) tested the effect of age of plants at the time of inoculation on symptoms, yield, and seed transmission. They noted that in Compana and Titan barley varieties early inoculations did not give symptoms in all plants, although assays showed that the virus was present in the seeds of some plants not showing symptoms. Inoculations at several stages from tillering through the soft dough gave symptoms in all plants of both varieties. The lowest yields were obtained from plants inoculated in the tillering stage, 40 days after planting. Titan was generally less affected by inoculations than was Compana. In work with wheat, Hagborg (7) found considerable reduction in plant height, grain yield, and quality from early inoculations but not from late.

During the summers of 1956 and 1957 field experiments were carried out with the same barley varieties as used in the greenhouse temperature studies. There were usually 10–12 spaced plants of each variety in

Table 2.—Effect of air temperature on tolerance of barley to stripe mosaic virus

Temperature (°C)	Disease indices[a] at given days after inoculation			
	Oderbrucker		C.I. 3212-1	
	10	20	10	20
16	17	36	0	2
20	18	42	8	11
24	24	51	8	16
28	43	58	14	32

[a] 0 = no symptoms in any plants; 100 = all plants killed. Individual plants rated according to severity of symptoms. Values shown are averages of 40–45 plants.

Table 3.—Effect of age of plant on symptom severity of stripe mosaic virus in barley field plantings

Plant age at inoculation (days)	Days after inoculation	Disease indices[a] in variety indicated				
		Oderbrucker	C.I. number			
			5020	3212	3212-1	4219
1956:						
20	15	20	5	7	7	7
	30	34	17	14	14	14
30	15[b]	12	5	3	3	2
	30	12	1	1	1	2
1957:						
20	15	27	15	14	12	9
	30	68	40	43	35	23
30	15	56	33	20	21	19
	30	65	47	35	32	42
40	15[b]	46	27	29	33	27
	30	54	35	37	36	45

[a] 0 = no symptoms in any plants; 100 = all plants killed. Individual plants rated according to severity of symptoms. Values shown are respective averages of 6 and 3 replications for 1956 and 1957.

[b] Indicates appearance of yellow-dwarf in oats and barley.

a plot. Plots were bordered by oat plantings, which served to delineate the plots and to indicate occurrence of the barley yellow-dwarf virus. From time to time, plants were sprayed with Malathion or Systox to minimize the incidence of the yellow-dwarf virus.

First inoculations were made at the 2–3-tiller stage, about 20 days after planting; subsequent inoculations were at intervals of 10 days until kernels of the plants reached the dough stage. In the first 3 inoculations the lower leaves of plants were inoculated individually. In later inoculations the entire plants were rubbed with the inoculum. This technique facilitated inoculation of plants at different stages of growth with greater uniformity of infection. Inoculated plants were rinsed with water immediately after inoculation. Infections were 90% or more in nearly every case. Each experiment was replicated 2–3 times, and symptom observations were recorded 15 and 30 days after inoculation. In the first 3 inoculations, each plant was rated by the disease index method described above. It was difficult to rate the individual plants in the later inoculation series since infected plants had produced several tillers. The appearance of yellow-dwarf in the plots further complicated the rating for stripe mosaic. Under these conditions, varieties were graded on the basis of symptoms expressed by groups of plants in a row, rather than on symptoms shown by individual plants.

The average growth period of barley varieties was about 75–80 days. Uninoculated plants included as controls remained free of stripe mosaic infection.

Oderbrucker plants were equally susceptible at all stages of growth. Plants inoculated 20 days after planting developed severe mosaic symptoms, later followed by apical necrosis. The newly produced tillers from plants that survived were also infected, but symptoms were less severe and some fertile heads were produced. In the later inoculations, the mortality of infected plants was much lower. Symptoms were more conspicuous on plants inoculated 30 days after planting than on plants inoculated at later stages of growth. Tillers arising from such infected plants showed the most severe symptoms, and symptom expression on them was greatly prolonged. In the last two inoculations, made 50 and 60 days after planting (heading and soft-dough stages), young tillers that developed later did not show symptoms.

Disease development in resistant and susceptible varieties, inoculated at different stages of growth, is compared in Table 3. It is evident that symptoms were more severe if plants were inoculated before they reached the boot-leaf stage (about 40 days after planting). The tolerant varieties were susceptible to infection at all stages of growth, but symptoms were moderate in comparison with Oderbrucker, and no leaf necrosis occurred. Initially, 3212-1 and 4219 appeared to be more resistant than 5020 and 3212, but at the heading stage, symptoms were as severe on 4219 as on 5020 and 3212. At this stage, 3212-1 was more resistant than all others.

These results suggested that the barley varieties under study were susceptible to infection at all stages of growth under field conditions, but that symptoms were most severe when plants were infected before they reached the boot-leaf stage. In comparison with Oderbrucker, other varieties showed moderate to high tolerance at all stages of growth.

Effect of age of plant and air temperature on seed-transmission.—It has been shown by Eslich and Afan-

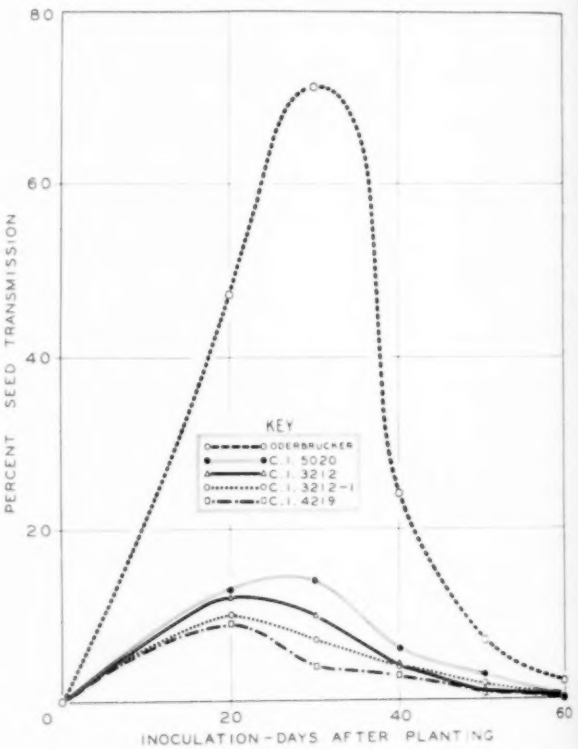

Fig. 2.—Effect of age of plant at time of inoculation on seed-transmission of barley stripe mosaic virus in several barley varieties.

Table 4.—Effect of age of plant on seed-transmission of barley stripe mosaic virus in 2 successive generation barley crops, I_1 and I_2 (1956 inoculation)

Plant age at inoculation (days)	Percentage seed transmission[a]							
	C.I.5020		C.I.3212		C.I.3212-1		C.I.4219	
	I_1[b]	I_2[c]	I_1	I_2	I_1	I_2	I_1	I_2
20	6	5	9	3	8	2	4	4
30	8	3	3	2	2	1	3	2
40	2	3	1	1	1	1	0	1
50	1	1	1	1	2	1	0	0
60	6	0	2	0	0	0	0	0

[a] Values shown are averages of 2 assays.
[b] Indicates plants (I_1) infected through mechanical inoculation. Seed harvested only from plants showing symptoms.
[c] Seed from plants of the next generation following inoculation. Seed harvested from all plants, whether they showed symptoms or not.

asiev (5) that under field conditions seed-transmission was highest when barley plants were inoculated in the boot stage with 63.7% of the seed infected in Compana and 4.4% in Titan. The difference between the varieties occurred in spite of the fact that plants of both varieties appeared to be equally susceptible. In wheat, Hagborg (7) found that early inoculation gave seed-transmission while inoculation at the time the plants were headed gave no transmission. In the present work, similar tests were made with the barley varieties being used.

Relation of age of plant to seed-transmission.—It was evident that the tolerant barley varieties were susceptible at all stages of growth, but symptoms on them were moderate in comparison with those on Oderbrucker. It was of interest to see if the amount of seed-transmission in these varieties was correlated with symptom severity.

In the two field experiments in 1956 and 1957, composite seed samples were obtained from plants inoculated at different stages of growth. Only plants that showed definite symptoms of virus infection were sampled. Seeds were assayed in the greenhouse as described by Afanasiev (1). In each assay there were 100 seeds per variety for each inoculation series and two or more replications. The percentage of seed-transmission was recorded 10–12 days after planting. In all experiments, seeds from uninoculated plants were included as controls. The results for a representative trial are plotted in Fig. 2. In the tolerant varieties, seed-transmission was maximum when plants were inoculated 20–30 days after being planted. In subsequent inoculations the percentage of seed-transmission dropped off. Oderbrucker showed a much higher rate of seed-transmission than did the tolerant varieties, with a maximum when inoculated 30 days after planting.

There was remarkable agreement with the results obtained earlier by Eslick and Afanasiev (5), both as to symptom severity and amount of seed-transmission. In Wisconsin, plant development of the highly susceptible variety was accelerated, probably because of the relatively late planting date.

It was of further interest to find out whether the frequency of seed-transmission in the second generation (I_2) of plants would be different from that of parental plants (I_1) that were mechanically inoculated. Seeds from mechanically inoculated plants were planted in the field and allowed to grow to maturity. Composite seed samples (I_2) from all plants, infected and uninfected, were assayed in the greenhouse as described above. The data (Table 4) show that the percentage of seed-transmission from plants infected through the seeds (I_2) was only slightly lower than that obtained from mechanically inoculated plants (I_1). Great reductions would be expected from the bulking of seed of infected and uninfected plants. This would be offset, more or less, by mechanical spread between plants in the field. From these data it would be expected that the rate of seed-transmission in varieties carrying this tolerance would never build up under field conditions.

Air temperature and seed-transmission.—In the foregoing experiments it was indicated that air temperature had a marked influence on the resistance of barley varieties 5020, 3212, 3212-1, and 4219. At cool temperatures, 16–20°, they were moderately to highly resistant in comparison with susceptible Oderbrucker. When exposed to warm temperatures, 24–28°, they all became moderately susceptible. To see if air temperature had a similar influence on seed-transmission of the virus in these varieties, seeds from plants grown at different temperatures were assayed for the virus, as described above.

Table 5.—Influence of air temperature on seed-transmission of barley stripe mosaic virus in resistant barley varieties

Temperature (°C)	Percentage seed transmission[a]			
	C.I.5020	C.I.3212	C.I.3212-1	C.I.4219
16	3	0	0	0
20	15	27	15	9
24	28	14	15	7
After temperature reversal				
16[b]	9	2	12	9
20[c]	2	8	5	10
24[d]	11	0	11	5

[a] Values shown are results of 2 trials for continuous 16 and 20°C and reversal 16°; one trial only for others.
[b] Held 12 days at 28°, then at 16°.
[c] Held 12 days at 24°, then at 20°.
[d] Held 12 days at 20°, then at 24°.

Infected Oderbrucker plants grown in the greenhouse usually failed to produce viable seeds at any of the temperatures. The resistant varieties yielded the maximum number of seeds at 20°. At 24°, the seeds obtained were poorly developed and were few in number. None of the varieties produced seeds at 28°. Varieties 3212 and 3212-1 headed much later than did 5020 and Oderbrucker, and 4219 was intermediate. When temperature conditions for plant growth were reversed by moving infected plants from 16°, 20°, 24°, and 28° to 28°, 24°, 20°, and 16°, respectively, all varieties produced seeds in accordance with the final temperature conditions.

Data for seed-transmission in resistant lines grown at different air temperatures are presented in Table 5. When infected plants were grown at 16°, only 5020 gave seed-transmission. At 20° and 24°, some transmission was obtained in all varieties, but since seed numbers were not large, the differences between the two temperatures are probably not significant.

In the temperature reversal series it was shown that an initial period of 12 days at 28° allowed the virus to invade the seeds even though the plants were returned to the 16° temperature for the remainder of the growth period. Temperature reversal at 20° and 24° had no noticeable effect.—Department of Plant Pathology, University of Wisconsin, Madison 6.

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THE NATURAL OCCURRENCE OF TOBACCO RINGSPOT VIRUS

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SUMMARY

Tobacco ringspot virus (TRSV) was tested for in 454 plants of 37 species from an area adjoining a field of soybeans where bud blight was epidemic. Host reaction and serological tests indicated that TRSV was yielded by 6 species that were symptomless (*Ambrosia artemisiifolia, Daucus carota, Erigeron strigosus, Rumex acetosella, Taraxacum officinale, Trifolium repens*) and 2 species showing symptoms (*Melilotus* spp. and *Trifolium pratense*). Attempts at mechanical infection of 5 of the 8 above-mentioned natural hosts were largely negative. Of seed from infected dandelion, 9–36% produced plants infected with TRSV (symptomless); no seed transmission was found in *E. strigosus*.

Tobacco ringspot virus (TRSV), which causes a severe disease of soybean (*Glycine max* (L.) Merrill) called budblight (1), is transmitted by seed of this host (4), though seed transmission appears of little importance in epidemics (2). A potential source of inoculum—plants bordering a field of heavily infected soybean, previously described (2)—was indexed for TRSV in 2 successive years, and seed transmission was

Table 1.—Numbers and kinds of hosts collected from the field and tested for the presence of TRSV

Species yielding TRSV	Symptoms on bean and cowpea	Symptoms on soybean and tobacco	Reaction with TRSV antiserum
Ambrosia artemisiifolia L.[a]	2/25[b]	−[c]	−
Daucus carota L.[a]	1/5	−	−
Erigeron strigosus Muhl.[a]	2/21	+[d]	+
Melilotus spp. (white & yellow sweet clover)	1/21	−	−
Rumex acetosella L.[a]	1/12	+	+
Taraxacum officinale Weber[a]	7/37	+	+
Trifolium pratense L.	1/49	+	+
T. repens L.[a]	1/32	+	+

[a] Not previously reported as a natural host of TRSV.
[b] Numerator indicates number of plants that yielded TRSV, denominator the number collected from the field and tested for TRSV.
[c] Not tested.
[d] At least one isolate from the different hosts was tested.

studied in 2 weed hosts. A preliminary report has been published (3).

Methods.—Juice from fresh tops of the plants were rubbed onto 3–4 plants each of bush bean (*Phaseolus vulgaris* L. var. Bountiful) and cowpea (*Vigna sinensis* (Torner.) Savi. var. Blackeye). In the second year the field plants sampled were marked by stakes and, if transmission was positive, were resampled. A number of isolates tentatively identified as TRSV were inoculated to *Nicotiana tabacum* L. (var. Wis. Havana Seed. No. H423) and soybean var. Harosoy, and a representative isolate was further tested on 12 hosts for verification. Centrifuged sap from artificially infected soybean was examined (by Dr. J. B. Bancroft) by ring precipitin tests against TRSV antiserum (kindly supplied by Dr. Ellen L. Moorhead).

Results.—*Isolation and identification.*—Sixteen isolates from 8 species (Table 1) appeared to be TRSV in the reactions of bean and cowpea. Species not yielding TRSV were *Ambrosia trifida* L., *Cirsium vulgare* (Savi.) Tenore, *Datura stramonium* L., *Lepidium virginicum* L., *Medicago sativa* L., *Plantago lanceolata* L., *Plantago rugelii* Dcne., *Polygonum aviculare* L., *Polygonum pennsylvanicum* L., *Pontentilla norvegica* L., *Rumex crispus* L., *Trifolium hybridum* L., and 17 other species tested in few numbers. The isolate from red clover produced symptoms similar to those described for TRSV (1, 10, 12) on *Apium graveolens* L., *Datura stramonium* L., *Glycine max* (L.) Merr., *Nicotiana tabacum* L., *Solanum carolinense* L., *S. nigrum* L., *Trifolium incarnatum* L., and *T. pratense* L. Symptoms on 2 previously unreported hosts for TRSV were: local lesions on *Amaranthus tricolor* L. var. *splendens* Bailey, and mild green spots and streaks on Alsike clover. Species not showing symptoms in 2 trials were: *Capsicum frutescens* L., *Solanum melongena* L. var. *esculentum*, *Vicia faba* L., and *Medicago sativa* L., which last has erroneously been reported susceptible (9) because (personal communication from Dr. K. M. Smith) of a mislabeled photograph (10). Of the symptomless plants, only egg plant yielded TRSV upon being indexed to bountiful bean. A positive reaction against TRSV antiserum confirmed the identity of the red clover isolate. Seven other isolates (all that were tried) also reacted with antiserum and produced diagnostic symptoms on soybean and tobacco (Table 1). The majority of the plants infected with TRSV in the field were symptomless. A red clover plant showed mild vein clearing, and one sweet clover plant had faint ringspots.

Mechanical inoculation of natural hosts.—Using different isolates of TRSV, various attempts were made to infect dandelion, *E. strigosus*, red, white, and ladino clover, and *Daucus carota* L. (5 of the 8 species found naturally infected with TRSV). Ten to 30 plants were mechanically inoculated, and indexed 3–6 weeks later. The result was no transmission to 3 species and limited infection of *E. strigosus* and red clover. The infected *E. strigosus* plant (1 of 29 inoculated) was symptomless.

Seed transmission.—Progenies of infected dandelion plants showed no virus symptoms. Thus each plant was indexed separately on bountiful bean plants, as were the progenies of healthy plants. The results (Table 2) show that dandelion may be added to the list of plants—tobacco (11), soybean (4), and petunia (6)—that transmit TRSV through their seed. Seed transmission may account for the prevalence of TRSV in dandelion.

Other viruses isolated.—Two viruses isolated from both sweet clover and white clover appeared similar

Table 2.—Seed Transmission of TRSV in dandelion and *Erigeron strigosus*

Source of seed	Results of indexing	% transmission
Infected dandelion No. 1	5/35[a]	14
Infected dandelion No. 1[b]	3/31	9
Infected dandelion from progeny of No. 1	12/41	29
Infected dandelion from progeny of No. 1	10/28	36
Healthy dandelion from progeny of No. 1	0/17	0
Healthy dandelion from progeny of No. 1	0/7	0
Healthy dandelion from progeny of No. 1	0/7	0
Infected *E. strigosus*	0/6	0
Infected *E. strigosus*	0/63	0

[a] Numerator indicates the number of plants that yielded TRSV when indexed on bountiful bean, denominator the number tested.
[b] In this repeated trial, seeds were collected 8 months after the preceding sample.

to TRSV on either bean or cowpea, but not on both. One was a pod-distorting strain (5) of yellow bean mosaic; the other resembled white clover mosaic (13) and severe pea mosaic (7). These viruses produced no budblight symptoms on soybean and could also be distinguished from TRSV by the reactions of a number of other hosts.

Discussion.—A number of previously unknown hosts naturally infected by TRSV are reported in this study, and at least one of them, dandelion, transmits the virus through its seed. Most of the natural hosts found (almost exclusively biennials or perennials) yielded virus before the middle of June, making them excellent primary sources of TRSV for soybeans. Because of the high incidence of natural hosts found, the list of wild hosts may be much longer. Reference to the large experimental host range (9) did not allow an accurate prediction of naturally infected hosts. A number of hosts reported to be susceptble to mechanical inoculation (9)—alsike clover, *Ambrosia trifida*, *Plantago lanceolata*—yielded no TRSV when collected from the field. Larger samples and improved isolation techniques, however, may make possible the detection of TRSV in these and other hosts. Conversely, when inoculated mechanically, 4 of the 6 natural hosts of TRSV were not infected and 2 species were infected in very small numbers. Thus, isolation from plants in the field is prerequisite to determining likely sources of the virus. The number and kind of plants infected in the field was probably determined by the host-vector relationships and the ability to transmit the virus through the seed, not by susceptibility to mechanical inoculation. There is now no effective control of budblight of soybean (8), a potentially important disease. It seems that eliminating, or at least decreasing the numbers, of natural hosts near soybeans would help control the disease. This could be accomplished with suitable herbicides applied to fence rows, grassways, etc., at planting time and by using clean culture methods. Other promising management practices would be to avoid planting soybeans at the edge of fields or next to pastures.

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COMPARISON OF CULTURAL VARIANTS OF ALTERNARIA SESAMI

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SUMMARY

A serious *Alternaria* disease of sesame in the United States is described. Isolates were found to differ markedly in virulence and cultural characteristics. The isolates were studied in relation to host resistance and taxonomic relationship. Duration of the moisture period following inoculation affected disease intensity, and 3-day periods resulted in considerable disease damage on the relatively resistant sesame strain Venezuela 51. No evidence of pathogenic specialization was found in tests involving 10 isolates and 11 sesame strains. Isolates varied in ability to reduce seedling emergence, but this reduction was not related to pathogenicity on foliage. Isolates showed no specificity for different sesame strains in their attack on germinating seed. The isolates in culture showed differences in appearance, growth rate, and could be separated into 5 groups with optimum temperature for growth of 20, 22.5, 25, 27.5, and 30°C. Spores of all the isolates were borne singly or occasionally in groups of 2 or 3 and were relatively similar in morphology. Sesame isolates were nonpathogenic on tomato, potato, muskmelon, watermelon, and several other crop plants. Pathogenic isolates of *A. solani* from tomato and potato, and *A. cucumerina* from muskmelon and watermelon were nonpathogenic on sesame. On the basis of these studies all the isolates were determined to be cultural variants of *A. sesami* (Kawamura) Mohanty & Behera.

Alternaria leaf- and stem-spot of sesame, *Sesamum indicum* L., is a hazard to production of this crop in the southeastern United States. Epiphytotics of this disease have been associated with excessive rainfall and high humidity (5). An Alternaria leaf-spot of

S. orientale was first described by Kvashnina (6), in North Caucasus. Kawamura (4) in Japan studied a similar leaf-spot pathogen on *S. indicum* and named it *Macrosporium sesami* Kawamura. Recently, Mohanty and Behera (7), in India, reported on an Alternaria blight of sesame and found the causal organism to closely resemble *M. sesami;* however, it differed from it in that some of the spores were catenulate. On the basis of catenulation, the fungus was placed in *Alternaria* and renamed *A. sesami* (Kawamura) Mohanty & Behera.

Information on the nature of the causal organism as it occurs in the United States is lacking and where referred to (5, 9) has only been designated as an undefined species of *Alternaria.* In the field, symptoms of the disease are brownish-red annular lesions on the cotyledons of emerging seedlings. Lesions are found to develop on the lower true leaves and then on the upper leaves. Under epiphytotic conditions, leaf drop may be premature and accompanied by lesions on the stem and capsules.

Development of commercial varieties with suitable resistance has been important in the improvement of this crop in the United States. Yearly fluctuation of disease intensity in the field has hampered testing for resistance. This study was carried out in conjunction with the development of methods for screening sesame strains for resistance in the greenhouse.

Materials and methods.—Pathogenicity of isolates initially made from sesame was determined by inoculation of the sesame variety Kansas 10. This variety has shown very low resistance in field trials. Plants were grown in the greenhouse at 75-90°F in a steam-sterilized 2:1 mixture of soil and vermiculite.

Cultures were grown in Petri dishes on Difco PDA (potato-dextrose agar) media. The inoculum for each isolate was prepared by homogenizing 2-week-old cultures with 100 cc of tap water in a Waring Blendor (1). The inoculum was sprayed onto the plants with a DeVilbiss atomizer attached to a compressed-air line. The plants were left 3 days in a moisture chamber in a greenhouse maintained at 65–75°F, except for tests in which disease intensity was studied in relation to time in the moisture chamber (1, 2, and 3 days). The plants were then removed from the chamber and placed on benches in the same greenhouse.

The pathogenicity of the isolates on germinating sesame seed was tested by covering the seed with inoculum prepared as previously indicated. The seed was then planted in steam-sterilized soil in 4-in. pots and germinated in a 70–80°F greenhouse. Each of the tests was carried out with 4 replicates and 50 seeds per replicate.

Isolations were made in 1956–57 from Alternaria lesions on the leaves and stems of sesame plants growing in test plots at Beltsville, Maryland. From several hundred isolates a group of 10 (A1 to A10) were selected that differed widely in pathogenicity and culture characteristics. In 1958, 15 (A11 to A25) pathogenic isolates were obtained from diseased foliage collected at Beltsville, Maryland, Florence, South Carolina, Gainesville, Florida, and Stoneville, Mississippi. All 25 pathogenic isolates were reisolated from Kansas 10 sesame inoculated in the greenhouse.

The 11 strains of sesame used in the pathogenicity studies represented a wide range of resistance and included several commercial varieties. They were as follows: Venezuela 51, Rio, Early Russian, Dulce, Kansas 10, Delco, Blanco, T53264-B-48-1-2-B, T53264-3-25-1-1-B, T53178-2-10-3-11-1-B, and B-1-8-6-2-1-2B. The latter 4 strains are respectively referred to herein as T1, T2, T3, and B1.

Isolate pathogenicity under field conditions was determined with the 11 strains of sesame in 2 plots separated by a 75-foot plot of Castorbeans. Five-foot rows of each of the 11 strains were replicated 4 times. Two plantings of each were made, on June 25 and July 18, 1958. On August 6, 1958, the plants in one plot were sprayed with a suspension of homogenized cultures of isolate A5; the other plot was left as a check.

In the greenhouse inoculations of 2- and 3-month-old sesame plants were carried out to determine possible pathogenic specialization of isolates A1 through A10. Five or more plants of each sesame strain were inoculated in each test with Alternaria isolates A1 to A10. Disease ratings were made 14 days after inoculation.

Optimal temperatures for growth were determined by inoculating Difco PDA medium in Petri plates with 0.5-cm-diameter portions from 2-week-old cultures. The isolates were grown 8 days at a series of temperatures with 2.5° increments from 10 to 35°C. Two plates of each isolate were grown at each temperature in 3 trials.

Spore measurements were made on spores collected from 2-week-old cultures. Spores from the host were collected from lesions produced on the foliage of Kansas-10 plants inoculated with the cultures 2 weeks before collection. The spores were mounted in lactophenol, on slides, for measurement.

Experimental results.—*Symptoms on sesame inoculated in the greenhouse.*—Damping-off occurred when seedlings were attacked by the *Alternaria* isolates. Dark-brown to black water-soaked lesions, followed by a characteristic shrinking of the tissues involved, occurred on the foliage and stems. The pathogen produced leaf spots up to 2 cm in diameter on older plants. In many instances the same isolates were found to produce lesions that were smaller and had dark necrotic margins in addition to annular lesions characteristic of the *Alternaria* (Fig. 2-A,B). The smaller lesions were generally found to be produced by the isolates possessing only moderate pathogenicity. Henning and Alexander (3) observed a similar range in the characteristics of lesions on several *Lycopersicon* species inoculated with *A. solani.*

Lesions on stems of sesame varied from single isolated lesions, sometimes sunken from tissue shrinkage, to spreading water-soaked lesions (Fig. 2-E). Lesions produced on the capsules were typically dark-brown and circular (Fig. 2-D).

Several tests were made to determine the effect on

Table 1.—Intensity of disease produced by 10 Alternaria isolates from sesame on the foliage of 11 different strains of sesame 14 days after inoculation in the greenhouse[a]

Sesame strain	Alternaria isolate										
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	Average
Venezuela 51	0.8	1.5	1.5	0.0	0.3	0.0	0.8	0.3	0.3	0.0	0.6
Rio	2.3	1.5	1.8	1.3	0.5	1.3	0.5	0.3	0.5	0.3	1.0
T1	3.8	2.3	2.5	0.8	0.5	0.5	0.3	0.3	0.5	0.0	1.2
T2	3.3	2.5	3.0	0.5	0.5	0.5	0.8	0.5	0.5	0.8	1.3
T3	2.5	3.8	3.8	2.5	0.8	2.3	1.5	0.5	1.0	0.5	1.9
Early Russian	3.8	4.0	3.5	3.0	1.3	2.8	1.0	1.0	0.8	2.8	2.4
Dulce	4.0	3.8	4.0	2.3	3.8	0.5	2.3	2.0	0.8	0.5	2.4
B1	4.0	3.8	3.3	3.5	3.3	2.5	1.0	1.5	1.0	0.3	2.4
Kansas 10	4.0	40	3.8	4.0	3.0	3.3	2.8	1.5	1.5	1.5	2.9
Blanco	4.0	4.0	3.8	4.0	3.5	2.5	2.8	2.0	2.3	2.0	3.1
Delco	4.0	4.0	3.8	4.0	3.5	3.3	3.0	2.3	1.8	2.5	3.2
Average	3.3	3.2	3.2	2.4	1.9	1.8	1.6	1.1	1.0	1.0	

[a] Average of 2 tests (first test on 2-month-old plants, second test on 3-month-old plants) with 5 plants of each sesame strain inoculated with each *Alternaria* isolate per test. Disease ratings: 0 = no lesions, 1 = occasional lesions, 2 = lesions sparse but uniformly distributed, 3 = dense uniform distribution of lesions, 4 = complete necrosis.

disease intensity of duration of the moisture period after inoculation. When Venezuela 51 and Kansas 10 were inoculated with Alternaria strain A5, good differentiation of resistance resulted with incubation periods of 1 day, 2 days, and particularly 3 days (Fig. 1). The prolonged moisture period resulted in some infection on Venezuela 51, indicating that Venezuela 51, at present one of the more desirable sources of Alternaria resistance for sesame improvement, would be expected to experience considerable damage under severe epiphytotic conditions.

Inoculation of sesame strains with Alternaria isolates.—Two inoculation tests were carried out on the 11 sesame strains with isolates A1 to A10. Though disease expression differed in the 2 tests, the relationships were the same. The isolates differed in virulence, but there was no evidence of pathogenic specialization (Table 1). There were marked differences in pathogenicity among the isolates on the foliage, stems, and capsules. The average disease intensity produced by each of isolates A1 to A10 on the 11 sesame strains demonstrated a close relationship between infection on foliage and infection on stems. Thus, isolate A1, besides producing on the foliage a greater disease intensity than that produced by A2 to A10, was similarly virulent on the stems. Likewise, isolate A10, which produced on the foliage less disease intensity than that produced by A1 to A9, was also mild on the stems. Infection by all isolates was more severe on the foliage than on the stems, and markedly more moderate on the capsules than on either the foliage or stems. The average disease intensity produced by isolate A1-A10 on the foliage, stems, and capsules of the 11 sesame strains was respectively 2.1, 1.4, and 0.4 (Table 2).

The individual sesame strains showed differences in susceptibility to the isolates, increasing in the following order: Venezuela 51, Rio, T1, T2, T3, Early Rus-

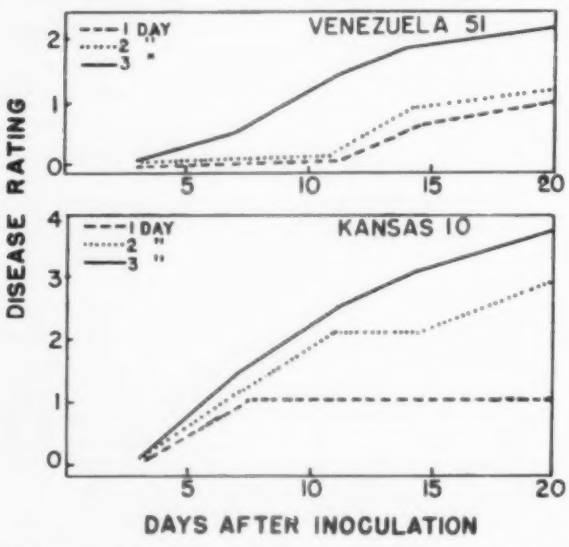

Fig. 1.—Effect of duration of the moist period (1, 2, and 3 days after inoculation) on the development of leaf spot on Venezuela 51 and Kansas 10 sesame inoculated with A5.

Table 2.—Intensity of disease produced by 10 Alternaria isolates from sesame on the foliage, stems, and seed pods of sesame 14 days after inoculation in the greenhouse[a]

Alternaria isolate	Foliage	Stems	Capsules
A1	3.3	1.9	0.5
A2	3.2	2.3	0.4
A3	3.2	2.7	1.3
A4	2.4	1.5	0.5
A5	1.9	1.5	0.5
A6	1.8	1.8	1.0
A7	1.6	1.1	0.6
A8	1.1	0.3	0.0
A9	1.0	0.4	0.0
A10	1.0	0.8	0.3
Average	2.1	1.4	0.4
LSD 5%	0.7	0.8	0.7

[a] Average of 2 tests (first test on 2-month-old plants, second test on 3-month-old plants) with 5 plants per test of sesame strains Venezuela 51, Rio, T1, T2, T3, Early Russian, Dulce, B1, Kansas 10, Blanco, and Delco. Disease ratings: 0 = no lesions, 1 = occasional lesions, 2 = lesions sparse but uniformly distributed, 3 = dense uniform distribution of lesions, 4 = complete necrosis.

sian, Dulce, B1, Kansas 10, Blanco, and Delco. The average disease intensity produced by isolate A1 to A10 on these 11 strains was, respectively, 0.6, 1.0, 1.2, 1.3, 1.9, 2.4, 2.4, 2.4, 2.9, 3.1, and 3.2 (Table 1).

Effect of Alternaria isolates on emergence.—Inoculations made at time of seeding showed that the isolates were capable of reducing the emergence of all 11 sesame strains tested. In 2 tests the uninoculated check of all 11 sesame strains had an emergence of 59%. The average emergence of all 11 inoculated

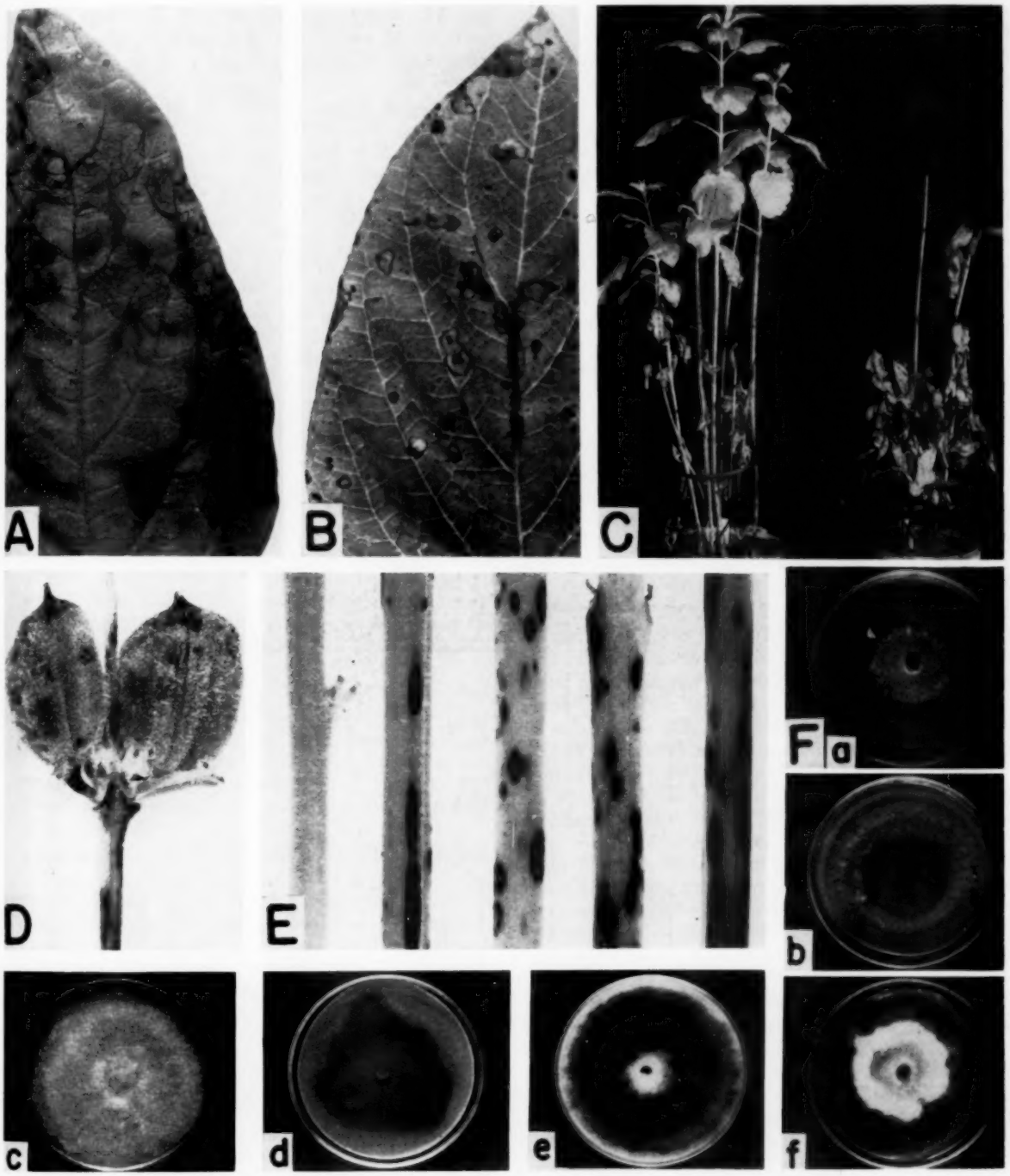

Fig. 2.—A, B) Portions of Kansas 10 sesame leaves infected with A7, from different tests. **C)** Venezuela 51 (left) and Kansas 10 (right) 3 weeks after inoculation with A5. **D)** Capsules of Kansas 10 infected with A3 (circular lesions). **E)** Portions of stems from Early Russian (uninoculated check) followed by those infected by A7, A4, A8, and A3, respectively. **F)** Cultures of *Alternaria* isolates illustrating the range of cultural types: a) A1, b) A3, c) A4, d) A6, e) A7, and f) A10.

sesame strains for seed inoculated with isolates A1 to A10 was, respectively, 32, 36, 45, 25, 23, 19, 29, 52, 30, and 9%. There was no relation between reduction in emergence and degree of pathogenicity as determined by foliage inoculation. Thus isolate A3, which showed a high degree of pathogencity on foliage, allowed an average of 45% emergence, compared with 59% for the control. Isolate A10, which was much less pathogenic than A3 in foliage inoculation tests, resulted in more severe emergence reduction, allowing only 9% average emergence.

In addition to a lack of relationship between reduction in emergence and pathogenicity there was no differential selectivity in the degree to which the individual isolates reduced the emergence of the 11 strains of sesame. As previously indicated, the average of all 11 sesame strains inoculated ranged from 9% for seed inoculated with isolate A10 to 52% for seed inoculated with isolate A8. Thus, for all 11 sesame strains—Venezuela 51, Rio, T1, T2, T3, Early Russian, Dulce, B1, Kansas 10, Blanco, and Delco—isolate A8 reduced emergence respectively by 0, 30, 6, 30, 0, 21, 16, 10, 10, 11, and 23%; isolate A10 reduced emergence respectively by 82, 88, 91, 86, 89, 79, 81, 75, 90, 89, and 96%.

Field test of pathogenicity.—A test showed that relative disease intensity on the 11 sesame strains was similar in the field to that produced by the same isolate on the 11 sesame strains in the greenhouse. The following average disease intensities were observed respectively for Venezuela 51, T1, T3, T2, Rio, Dulce, Blanco, Early Russian, B1, Kansas 10 and Delco: 1.0, 1.0, 1.0, 2.0, 2.5, 2.5, 2.5, 2.5, 3.0, 3.0, and 4.0. The uninoculated plot remained almost completely free of any disease, and the intensity of disease on the first and second plantings in this study was about the same.

Cross-inoculation tests.—Inoculations were made with each of isolates A1 through A25 onto plants in the true-leaf stage of each of the following: tomato (Chesapeake, Bonny Best and Pan American), castorbean (Baker 296, Dawn, Interspersed Dawn, Baker Hybrid 45, and 415 Hybrid), onion, beet, watermelon, muskmelon, squash, potato (Green Mountain and Katahdin), corn, cabbage, and zinnia. In most cases none of the sesame isolates produced any visible infection. Occasional pin-point necrotic lesions were produced on the lower, older leaves of the tomatoes and on the cotyledons of the castorbeans, though the causal organism could not be recovered.

Kansas 10 sesame was inoculated with the following *Alternaria* species: *A. solani* (Ell. & Mart.) Jones & Grout from potato and tomato, the pathogenicity of which was tested on Chesapeake, Bonny Best, and Pan-American tomato varieties; *A. cucumerina* (Ell. & Ev.) Elliott from muskmelon and watermelon, the pathogenicity of which was tested on Congo watermelon. The following *Alternaria* species, presumably pathogenic, from the American Type Culture Collection, Washington, D. C., were tested: *A. gossypina* (Thuem.) Hopk. and *A. radicina* Meier, Drechel. & Eddy. Several isolates of *A. tenuis* Auct. were also tested. None of these species appeared pathogenic on the sesame.

Cultural characteristics.—The optimal temperature for growth of the isolates in culture was found to range from 20 to 30°C. Some of the isolates differed in rate of growth. Thus, for those isolates whose optimal temperature for growth was 20°C, A17, A5, and A1, colony diameter after 8 days was respectively 49, 43, and 41 mm. A15 had an optimum growth temperature of 22.5°C and had a colony diameter of 90 mm after 8 days. The majority of the isolates had an optimum growth temperature of 25°C, which included A7, A13, A21, A25, A18, A22, A2, A20, A4, A14, A10, A11, A6, A12, and A23; colony diameter of these isolates after 8 days was respectively 90, 90, 90, 90, 87, 85, 76, 74, 73, 69, 61, 61, 57, 53, and 52 mm. Isolates A24, A8, and A9 had an optimum growth temperature of 27.5°C, and after 8 days respective colony diameters of 86, 69, and 62 mm. Isolates with the highest optimum growth temperature, 30°C, were A19, A3, and A16, and these had a colony diameter after 8 days of 80, 79, and 74 mm.

The isolates in culture exhibited wide differences in color and form of growth. Isolates A1, A3, A4, A6, A7, and A10 had cultural characteristics representative of the wide range of all 25 isolates (Fig. 2-F). A1 was medium flat with a brownish-black underlying

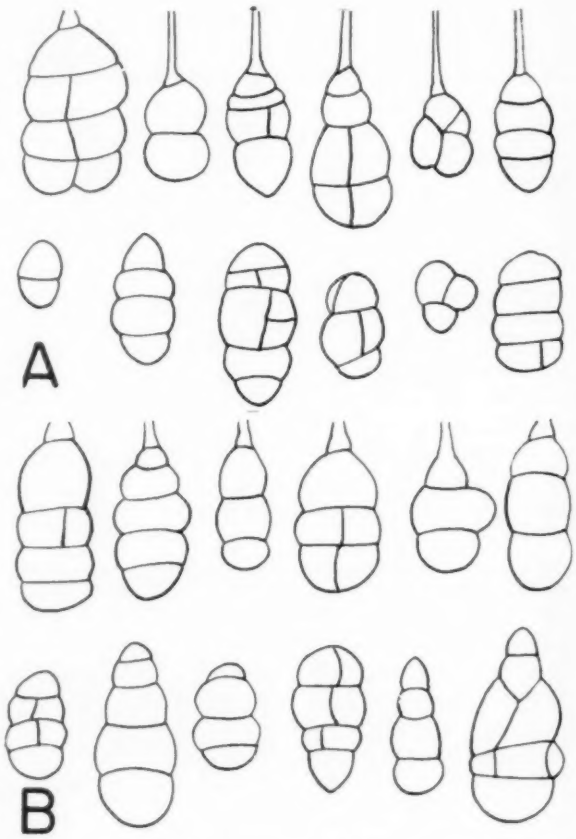

Fig. 3.—Spore morphology: **A)** A5, an isolate with relatively small average dimensions; **B)** A2, a strain with relatively large average dimensions (×840).

layer: the surface was loose cottony and white. A3 was medium high, dense cottony, dark gray, becoming dark-brown; there was some surface development of tufted dark-gray mycelium. A4 was medium flat, dense cottony, and gray; the surface was loose cottony and grayish white. A6 was flat, felt-like, and dark-brown; the surface was dense cottony and dark-gray. A7 was flat, cottony, and dark-gray; the surface was cottony and white. A10 was medium flat, dense cottony, and dark-gray; the surface was felt-like and white. Growth rate or culture type was not related to pathogenicity.

Spore characteristics.—Spores were borne singly in culture as well as on infected plant parts. Chains of 2 or 3 spores were formed occasionally. After repeated transfers several isolates lost their ability to sporulate in culture or to produce spores on the host. Thus isolates A3 and A8 lost their ability to sporulate in culture, and A2, A8, and A9 lost their ability to sporulate on the host. The spores were of 2 types: with filiform beaks and without. In general the transition between spore body and beak was abrupt (Fig. 3).

In culture, for spores with beaks, the range in the length of the average spore bodies was relatively small, 29–45 μ, as was also spore width, 12–19 μ. However, the range in average beak length was large, 62–369 μ. The average numbers of transverse walls in the spore bodies were in the range 2.1–4.1, and vertical walls in the range 0.0–1.3. From the host, for spores with beaks, average spore body length was 28–40 μ, and width was 11–15 μ; average beak length was 50–198 μ. The average number of transverse walls was 3.1–4.7, and number of vertical walls was 0.0–1.2.

In culture, for spores without beaks, average body length was 22–36 μ, and width was 12–18 μ. The average number of transverse walls was 2.5–5.0, and number of vertical walls was 0.2–3.3. From the host, average body length was 22–29 μ, and width was 10–15 μ; average number of transverse walls was 2.3–3.1, and number of vertical walls was 0.0–0.4.

Discussion.—On the basis of method of sporulation —in single units or occasionally in chains of 2 or 3 spores—all the isolates could be grouped with those *Alternaria* species classified by Neergaard (8) in the section Noncatenatae. However, this classification must be qualified since the spores were relatively small, many were pseudostemphyliumlike in form, and spores with beaks had a relatively abrupt transition from the spore body to the beak, characteristics more typical of the spore types found in Neergaard's section Brevicatenatae. The observation that occasionally the spores were catenulate would also make classification into the Brevicatenatae more appropriate.

The descriptions of pathogenic *Alternaria* on sesame from Russia (6), Japan (4), and India (7) all indicate the variability in the spore characteristics of the causal organism, particularly in regard to catenulation, as was observed in this study. Irrespective of the degree of catenulation, a characteristic whose variability has been well discussed in the literature on *Alternaria*, the recent reclassification of the pathogen from *Macrosporium* into *Alternaria* by Mohanty and Behera (7) is in accordance with the modern concept of this genus as reviewed by Groves and Skolko (2). This concept proposes the retention of *Alternaria* in its traditional sense and the discontinuance of the use of *Macrosporium*. Thus on this basis and the similarity in quantitative spore characteristics, the description of *A. sesami* agrees well with the *Alternaria* cultivars studied by the writer.

The variable and unstable nature of *Alternaria* pathogens is well known. That this Alternaria disease complex of sesame was found to be caused by a number of cultural variants is therefore as expected. In spite of the number of cultivars producing this disease complex, no pathogenic specialization was found. However, the isolates differed markedly in virulence, a condition that would require consideration in any controlled studies with this pathogen, such as screening sesame strains for resistance. In such resistance testing, period in the moisture chamber would also be important in the detection of different levels of resistance.

Venezuela 51 is currently used as a source of *Alternaria* resistance. This study demonstrated that the resistance of Venezuela 51 can be overcome by artificial inoculation. Likewise, it would be expected that if epiphytotic conditions in the field were sufficiently severe, Venezuela 51 would become heavily infected. Such was found to be true in 1958 on test plots at Tallahassee, Florida (personal communication from Dr. C. A. Thomas). Venezuela 51 and several closely related sesame strains were infected almost as severely as any of the relatively susceptible strains. Supplemental greenhouse tests provided evidence that leaf spot infection and consequent defoliation even at periods shortly before natural leaf drop, can markedly reduce seed yield. Thus, provided environmental conditions were not too favorable for the development of the pathogen, this resistance, even though intermediate, would be valuable in reducing yield loss in spite of some infection. Since inoculation conditions too severe could readily eliminate the highest resistance presently available, the utilization in a screening program of the different levels of pathogenicity as determined in this study, or severity of inoculation, would enable separation of the levels of resistance. Regulation of other variables such as plant age, inoculum age and concentration, and temperature should help increase the precision of such a test.—Crops Research Division, U. S. Department of Agriculture, Beltsville, Maryland.

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EFFECTS OF TEMPERATURE AND MOISTURE STRESS ON THE LETTUCE POWDERY MILDEW FUNGUS

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SUMMARY

The percentage of germination of conidia of *Erysiphe cichoracearum* (lettuce strain) was highest and growth of germ tubes and mycelia was most rapid at 18°C. The minimum temperature for infection and growth on lettuce (*Lactuca sativa*) was between 6 and 10°C and the maximum near 27°C. Over a 24-hr. period the most rapid germination occurred between 18 and 25°C. Moisture stresses giving the best germination of conidia appeared to lie in the very narrow range of 0.45–1.65 mm VPD (95.6–98.2% RH between 25 and 30°C). Germination of a small percentage of conidia occurred at 25 mm VPD (0.1% RH at 25°C). A humid atmosphere of 0 mm VPD (100% RH) inhibited germination. Development of powdery mildew appeared to be most affected by temperature. Atmospheric moisture, however, influenced the rapidity of disease development and disease severity.

Powdery mildew of cultivated lettuce (*Lactuca sativa* L.), incited by *Erysiphe cichoracearum* DC. ex Mérat, appeared in the United States in about 1951 (9, 10). Consequently, little was known until recently about the epiphytology, life cycle, host range, distribution, and sources of resistance (6, 8, 9). Some environmental factors affecting disease development were studied and briefly outlined by Deslandes (3) in 1954. The present writer, however, felt that a more detailed study was necessary of environmental factors affecting the fungus to improve understanding of the ecology and epiphytology of this powdery mildew. This paper presents the results of laboratory studies on temperature and moisture stress as they affect germination, infection, and growth of the lettuce strain of *E. cichoracearum*.

Observations and experimental results.—*Effect of temperature on germination of conidia.*—Preliminary work in the laboratory showed that the germination of conidia was influenced by several factors other than temperature: moisture conditions during germination, age of conidia, and physiological condition of the host on which the spores were produced. Spores were taken from detached lettuce leaves (var. Great Lakes) floated on distilled water in Petri plates, or from potted plants kept in a 12°C walk-in refrigerator. Relative humidity (RH) in the refrigerator was 60%. The infected leaves in both cases had received about 400 ft-c of illumination at the leaf surface. Spores were sampled from colonies 10–14 days old from detached-leaf culture and 21–28 days old from the potted plants. In any given experiment, a 300-spore sample was microscopically observed before inoculation to determine if there were any germinated conidia. Only samples with ungerminated conidia were used.

Glass slides were elevated above 10 cc of distilled water by two ⅛-in. glass rods in Petri dishes. The slides were dusted with conidia and the dishes placed in incubators at various temperatures (±0.5°C) for 48 hours. The effect of temperature on conidial germination was also studied in detached-leaf culture in Petri dishes. Celloidin strippings (2) were taken from lettuce leaves (var. Great Lakes) 48 hours after inoculation, and the spores were stained with cotton blue in lactophenol before observation.

In the majority of the experiments, percentage of germination was greater on detached leaves than on glass slides. The temperature range allowing germination was about the same with both substrates: minimum, 5°; optimum, 18°; and maximum, 34°C (Fig. 1). These results agree closely with those of Deslandes (3). The stimulation of germination on leaves was similar to that reported by Longree (4) in work with powdery mildew of rose. She suggested that microclimatic differences near the surface of the leaf might be responsible for germination differences between glass slides and rose leaves in detached-leaf culture, or that some stimulant peculiar to the leaf surface was responsible for a higher percentage of germination there. This possibility was studied, and its relationship to the development of lettuce powdery mildew will be discussed in a later paper on microclimate.

Effect of low temperature on viability of conidia.—The lettuce powdery mildew organism has been observed to overwinter in the conidial stage on old lettuce plants in the Salinas Valley (5, 6). It was not known, however, whether spores could survive in this way every year. A test was therefore performed to

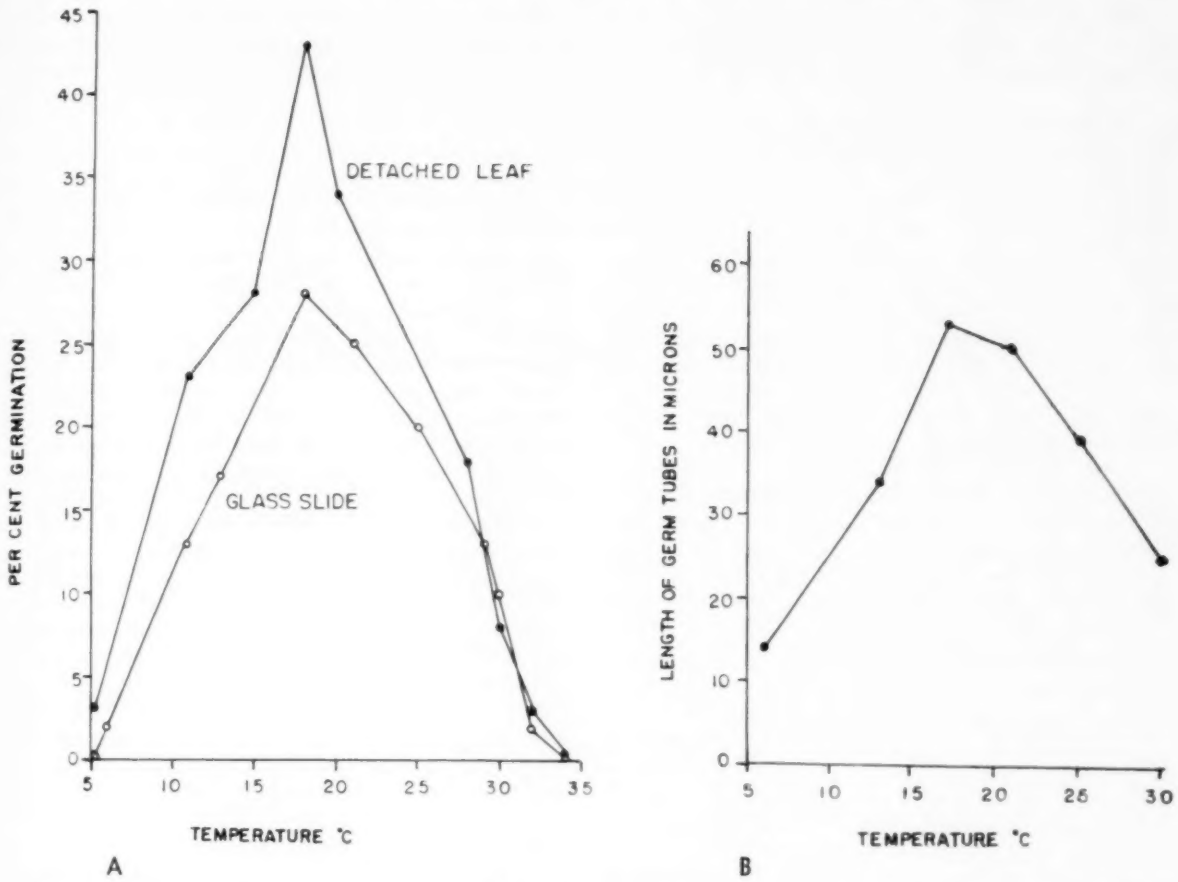

Fig. 1.—A) Percentage of germination of lettuce powdery mildew conidia on glass slides and on detached leaves at different temperatures after 48 hours of incubation. Each point of the glass-slide graph represents an average of 1800 spores, whereas each point of the detached-leaf graph represents an average of 900 spores. **B)** Length of germ tubes of lettuce powdery mildew on glass slides at different temperatures after 48 hours of incubation. Each point on this graph represents an average of 60 germ tubes

determine the effect of prolonged low temperature on the viability of conidia. Deslandes (3) reported that subjecting conidia of this pathogen to –10°C for 24 hours did not affect germinability. In the present study, spores were subjected to a constant –5°C on glass slides suspended in Petri dishes above 10 cc of distilled water. Samples of conidia were taken every 24 hours for a period of 168 hours, and germination percentages were determined after each sample was incubated for 48 hours at 18°C and 100% RH. Petri dishes containing glass slides dusted with newly formed conidia were incubated at 18°C and 100% RH for 168 hours and served as controls. Results (Table 1) showed that 3% of the conidia were still viable after a 168-hour exposure to —5°C. This test shows that conidia of the powdery mildew of lettuce can survive the coldest conditions likely to occur in the Salinas Valley, where temperatures rarely go below 0°C.

Effect of temperature on rapidity of germination.— The effect of temperature on rate of germination of conidia was also studied. Several circular cover glasses were fixed with white petrolatum to each of several microscope slides elevated above 10 cc of distilled water in Petri dishes. The cover glasses were dusted with spores, placed in incubators at various temperatures, and removed after different intervals over a 7-day period. Spores were stained with .05% cotton blue in lactophenol for immediate killing and observation. The following temperatures were maintained: 6, 13, 18, 21, 25, and 30°C. Maximum germination at a given temperature, as indicated by the last point plotted for each temperature in Fig. 2, was determined by averaging all germination percentages

Table 1.—Percentage of germination[a] of conidia of lettuce powdery mildew on glass slides after various periods at 2 temperatures

Exposure temperature (°C)	% germination after periods stated (hrs.)						
	24	48	72	96	120	144	168
-5	39	18	24	9	13	7	3
18 (control)	32	45	50	60	59	63	62

[a] Each figure is an average of 200 spores. After exposure to –5°C for the periods indicated spores were removed to 18°C and incubated 48 hours before percentage of germination of conidia was determined.

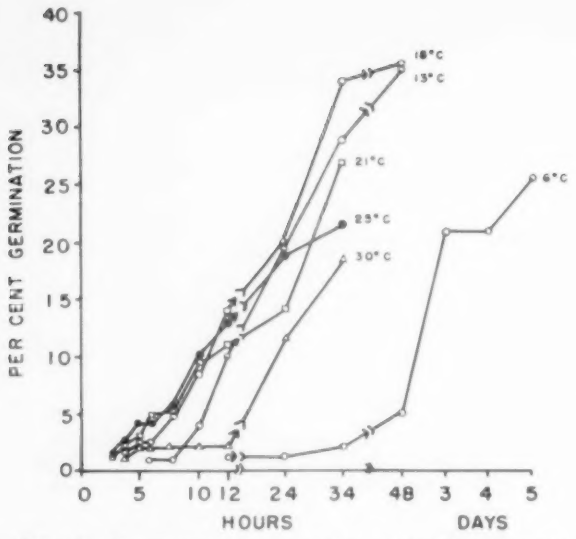

Fig. 2.—Percentage of germination of conidia of lettuce powdery mildew on glass slides at different temperatures after different periods. Each point on this graph through the 10-hour period is an average of 600 spores in 3 replications, whereas points from 12 hours to 5 days are an average of 400 spores in 2 replications.

occurring after the point of no increase had been reached during the 7-day incubation period.

The percentage of germination during the first 24 hours increased about as rapidly at 18 as at 25°C (Fig. 2). Germination occurred in 3 hours at 21 and 25°C, and in 4 hours at 18°C. After 24 hours, the percentage of germination increased more rapidly at 13 and at 18° than at 21 and 25°C, and after 48 hours the percentage of germination was about equal at 13 and 18°C. The rapidity of germination was considerably slower at 6°, at which temperature maximum germination was not reached until the fifth day. Percentage of germination was lowest at 30°, although rate of germination was faster than at 6°C.

Effect of temperature on growth.—The effect of temperature on growth of the organism was determined by measurements of germ-tube elongation on glass slides after a 48-hour incubation period at 6, 13, 18, 21, 25, and 30°C. In addition, the effect of temperature on the development of the whole colony was studied by examination of celloidin strippings (2) from detached-leaf cultures incubated at 5, 10, 15, 18, 21, 24, 28, 30, and 32°C.

The curve for the growth rate of germ tubes (Fig. 1-B) and increase in colony diameter followed the curve for germination very closely (Fig. 1-A) except that there was no growth beyond the primary germ-tube stage on detached leaves at 5 and 28°C. The optimum temperature for growth of germ tubes on glass slides and detached leaves and mycelia on detached leaves was 18°C. Germination, infection, growth, and sporulation proceeded normally, but at different rates, within the range of 10–28°C. These data are in agreement with those reported by Deslandes (3). At most, only half of the germinated spores infected detached lettuce leaves.

Effects of moisture stress on germination of conidia.—A 20-unit Warburg apparatus (Precision Scientific Co.) was used to study the effects of moisture stress on conidial germination. The 15-cc-capacity reaction vessels with a single side-arm were filled with 5 cc of humidity-regulating solutions composed of sulfuric acid and redistilled water. Stevens' tables (11) were used as a guide in making the humidity-regulating solutions. Small glass slivers dusted with conidia were attached to the side-arm stopper (2) by means of Vaspar (paraffin and vaseline) and inserted into the side arm. The water bath was maintained at 25±0.1°C. Spore germination was tested in duplicate at the following approximate RH: <0.1, 4, 9, 25, 48, 86, 93, and 100%. Since the germination of various spore lots was variable, fresh spores were germinated at 18°C and 100% RH in Petri dishes as a control in order to determine the germination potential of a given spore lot. The glass slivers containing the conidia were removed from the side arms of the Warburg reaction vessels after 24 hours, and germination percentages were determined.

Moisture conditions are expressed both as % RH and as moisture stress (mm of vapor pressure deficit, VPD). VPD has been shown to be more precise than RH as a measure of moisture stress (1, 2, 11), but in either case the temperature should always be given (2). VPD can be determined from psychrometric readings. In this study it was calculated by the formula VPD = (1 – RH)E, where E is the vapor pressure at saturation at a given temperature and RH is the relative humidity (11). VPD changes if

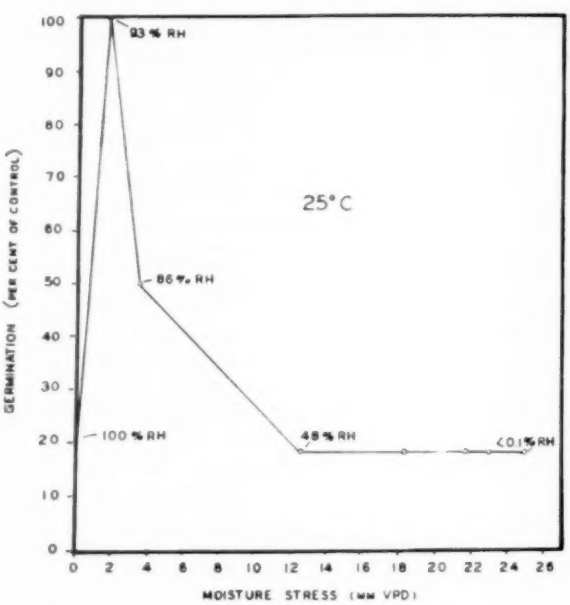

Fig. 3.—Percentage of germination of conidia of lettuce powdery mildew at different moisture stresses at 25°C after 24 hours of incubation. Each point on this graph is an average of 200 spores in 2 replications. Percentage of germination was calculated as the percentage of germination of the control conidia, incubated at 18°C and 100% RH.

temperature changes, even when RH remains constant.

Deslandes (3) reported that high RH (85%) was essential for conidial germination, infection, mycelial growth, and sporulation of the lettuce powdery mildew fungus.

Moisture stress markedly influenced the percentage of conidia that germinated, but germination percentage was small under conditions of severe moisture stress (Fig. 3). Germination decreased rapidly below 1.5 mm VPD at 25°C (93% RH). However, germination occurred below 13 mm VPD (48% RH) at a low but constant percentage (Fig. 3). Germ tubes that formed below 86% RH at 25°C (3.6 mm VPD) were abnormally short and somewhat thicker than those of spores germinated under control conditions (18°C and 100% RH). Most spores subjected to a RH lower than 86% (3.6 mm VPD) shriveled and did not germinate. The lower germination percentage at 100% RH than at 93% RH was probably due to condensation of moisture. This phenomenon has been reported previously for other powdery mildews (2, 4, 12, 13).

A series of high RH (85.7, 93.2, 95.6, 98.2, and 100%) was studied for more accurate determination of the critical range of moisture stress for germination. Several temperatures (25, 27, and 30°C) were tested to compare effects on the percentage of germination of similar moisture stresses at different temperatures. Tests were performed in duplicate in the Warburg apparatus. As a control, germination tests were made at 18°C and 100% RH in Petri dishes.

In every case condensation of moisture at 100% RH reduced germination. Percentage of germination was higher than that of the control at 25°C and 1.10 mm VPD (95.6% RH), as high as that of the control at 25°C at both 0.45 and 1.65 mm VPD (98.2 and 93.2% RH, respectively), but less than that of the control at 25°C and 3.5 mm VPD (85.7% RH, Fig. 4). Germination at 27°C was as good as that of the control at 0.51 mm VPD (98.2% RH), but dropped sharply below this point. Percentage of germination at 30° was highest at 0.60 mm VPD (98.2% RH).

These data show that percentage of germination of the conidia of this powdery mildew, in contrast to that of some other powdery mildews, is affected adversely by moisture stress. Condensation of moisture is also inhibitory. At the temperatures tested, moisture stresses giving the best germination appeared to lie in the very narrow range of 0.4"–1.65 mm VPD.

Discussion and conclusions.—The optimal temperature for germination and growth of the lettuce powdery mildew fungus is near the average temperature (18°C) where the disease is the most severe in the Salinas Valley (5). Since average temperatures are lower in the Salinas Valley than in some other lettuce-growing regions of California, temperature may be a factor limiting the severity of the disease in other areas. The appearance of powdery mildew north and south of the Salinas Valley is correlated with the build-up of the disease in the Salinas Valley in that the primary source of inoculum for infections in other areas appears to be the Salinas area (6, 9). Consequently, wind direction and velocity may also be important in determining the distribution of this disease (5, 6).

These studies show that conidia of this fungus are capable of surviving temperatures much lower than those occurring in the Salinas Valley during the winter. Even though the perfect stage has been shown to play the major role in the overwintering of this parasite (6) it is apparent from observations (6) and evidence on temperature relations obtained in this study that the fungus can overwinter in the asexual stage. The importance of the asexual stage as the primary source of inoculum for the spring and summer crops appears to depend on the prevalence of overwintering lettuce plants with mildew infections at the time when the young lettuce becomes susceptible (7).

The Salinas Valley is characterized by a higher RH and lower VPD than most lettuce-growing regions in California. Tests on lettuce plants in greenhouse and laboratory (RH 60% or lower) showed that development of the fungus at favorable temperatures is poor if moisture stress (VPD) is high. This appears to be caused by reduced germination of conidia and not by decreased growth of mycelia or failure of the fungus to produce spores. If VPD is low the percentage of germination of conidia is high and the disease is much more severe, because of greater density of mildew growth. Atmospheric moisture, thus, does not appear to determine the presence or absence of the disease under conditions found in California lettuce-growing areas, but markedly influences the rapidity of development and severity of the disease.

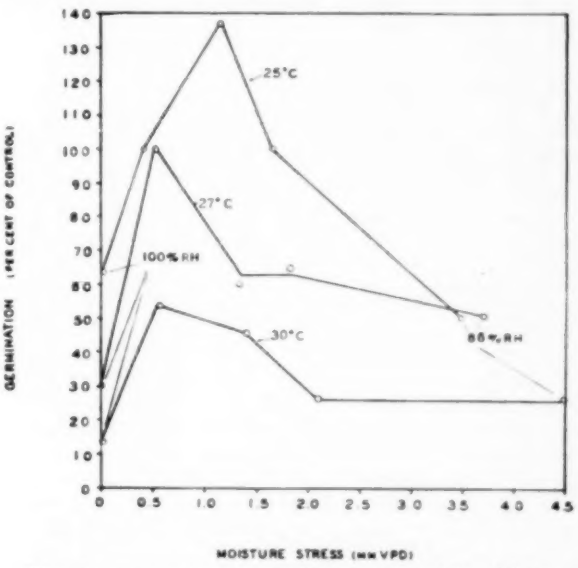

Fig. 4.—Percentage of germination of conidia of lettuce powdery mildew at different moisture stresses at different temperatures after 24 hours of incubation. Each point on this graph is an average of 400 spores in 2 replications. Germination was calculated as percentage of the germination of the control conidia, germinated at 18°C and 100% RH.

It appears, however, that temperature is of more importance than VPD in determining the ecology of this disease.

In these studies, the maximum percentage of germination of conidia was reached in 2 days at optimal or near-optimal temperatures; however, germination usually continued for 3–4 days at optimal temperatures in other experiments, e.g., control treatment (Table 1). Atmospheric moisture levels favorable for a high percentage of conidial germination exist for about 12–14 hours (about 8:00 P.M.–8:00 A.M.) each day in the Salinas Valley (5). Therefore, establishment of the disease would appear to be markedly influenced by temperature during this period of favorable atmospheric moisture.

It appears from the present study and the studies of others (2, 4, 12, 13) that powdery mildews fall into 3 categories in regard to the effects of atmospheric moisture on conidial germination. These categories are as follows: 1) powdery mildews that show little or no response to atmospheric moisture conditions, 2) powdery mildews that germinate best at high RH, but are capable of some germination at very low RH, and 3) powdery mildews that germinate only at high RH. Since conidial germination of most if not all powdery mildews is inhibited by condensation of moisture, this condition is not taken into account in the above categories. The lettuce powdery mildew fungus falls into the second category.—Department of Plant Pathology, University of California, Davis.

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WILD SOURCES OF BLUEBERRY STUNT VIRUS IN NEW JERSEY

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SUMMARY

Surveys indicated that blueberry stunt is widespread through the native population of *Vaccinium vacillans* in all areas of New Jersey. Stunt was also found affecting wild *V. corymbosum*, *V. atrococcum*, and *V. stamineum* throughout the range of these species in New Jersey. The virus was transmitted from wild species to cultivated *V. corymbosum* var. Jersey by grafting, and to *Vinca rosea* by dodder, *Cuscuta subinclusa*. The widespread distribution of stunt in wild species of *Vaccinium* in New Jersey suggested that the virus is indigenous and was spread by its vector into the first cultivated blueberry fields.

In 1942 Wilcox described stunt of cultivated blueberries, *Vaccinium corymbosum* L., and showed that the disease was graft-transmissible (11). Tomlinson et al (10) found that the virus was transmitted to healthy blueberries by a mixed colony of leafhoppers. Hutchinson (5) and Maramorosch (7) later showed that *Scaphytopius magdalensis* Prov. is the principal vector.

Although Wilcox (11) did not say that stunt affected wild *V. corymbosum* near blueberry plantings in New Jersey, stunt was later so observed within a mile of cultivated fields (4). Some believed that stunt was brought into New Jersey in seedlings grown at Washington, D. C., and that the virus spread to wild plants from cultivated fields (4). However, observation by the senior author of stunt-like symptoms on wild *V. corymbosum* in the Pocono Mountains of northeastern Pennsylvania in 1950 suggested that stunt is an indigenous disease. Stevens (9) as early as 1926 reported a wild blueberry with virus-like symptoms.

In 1924 Stevens (8) remarked that "With the blueberry, which is just now coming into cultivation, we feel that we have an opportunity perhaps unique in

Table 1.—Transmission of stunt virus from wild plants of Vaccinium species growing near New Brunswick, New Jersey

Species tested	Number of plants tested[a]	Indicator plant	Method of transmission	Number of transmissions	
				Attempted	Obtained
V. atrococcum	5	Jersey[b]	Chip budding	10	5
		Jersey	Whip grafting	9	6
V. corymbosum	6	Jersey	Chip budding	14	7
		Jersey	Whip grafting	10	6
		Vinca rosea	Dodder	3	2
V. stamineum	1	Jersey	Chip budding	2	1
		Jersey	Whip grafting	8	2
V. vacillans	3	Jersey	Whip grafting	8	2
		Vinca rosea	Dodder	7	1

[a] Virus was found in all plants tested by 1 or more of the transmission techniques.
[b] A cultivated variety of highbush blueberry, *V. corymbosum*.

our time, for studying the progress of diseases in a native wild plant as it is brought under cultivation." The origin of the first cultivated blueberries has been described in considerable detail (2). The present paper presents evidence that stunt probably originated in wild blueberries and is indigenous to New Jersey. A preliminary report has been published (6).

Symptoms.—A characteristic symptom on cultivated and wild *V. corymbosum* is a yellowing of the leaves along the margins and between lateral veins (Fig. 1-F,G,H). Normal green is generally retained along the mid-rib and lateral veins, and affected leaves are often cupped and reduced in size. Chlorotic areas generally turn a brilliant red in early fall, and this color is distinctive until the development of normal fall coloration. Diseased plants live for many years, but are much reduced in vigor. Branches may continue to set some fruit, but the berries remain small and of poor quality. If a diseased plant is cut back to the ground, the new growth is weak and stunted. Branches are twiggy in appearance as a result of shortened internodes and the growth of normally dormant buds. So far as it is known, all cultivated varieties are susceptible, but some are more severely affected than others. The Rancocas variety, for example, seldom shows definite symptoms and produces apparently normal yields.

Symptoms on *V. vaccillans* Torr., *V. atrococcum* Heller, and *V. stamineum* L. are in general like those for highbush blueberry. On severely affected leaves of *V. vacillans*, normal green may be limited to a small portion along the midrib. Watersoaked areas have been observed in the spring on the expanding leaves of *V. vacillans* and *V. stamineum*. *V. vacillans* plants are often severely stunted and have been found less than 3 in. high, with tiny leaves and a compact growth habit (Fig. 1-A,B). Healthy plants are normally 6–18 in. high. Infected wild plants of *V. atrococcum*, *V. stamineum*, and *V. vacillans* generally set less fruit than do infected wild plants of *V. corymbosum*.

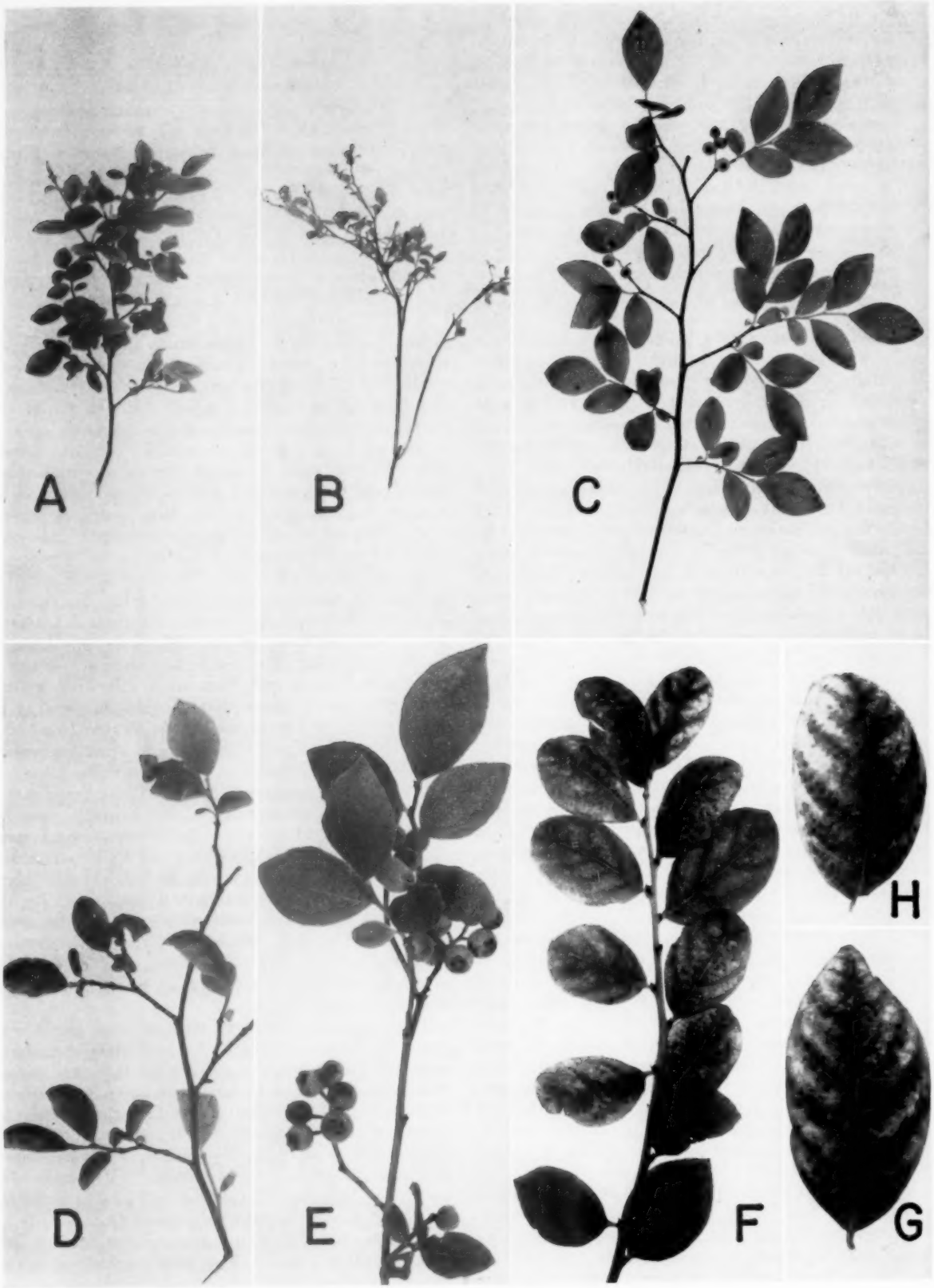
Methods.—Although at least 10 *Vaccinium* species grow wild in New Jersey, *V. corymbosum*, *V. vacillans*, *V. atrococcum*, and *V. stamineum* were the only ones included in the survey for stunt conducted in 1952–56. *V. australe* Small and *V. pallidum* Ait. are not readily distinguished from *V. corymbosum* and *V. vacillans*, respectively; *V. caesariense* Mackenz. and *V. augustifolium* Ait. have shown no recognizable symptoms; and *V. myrtilloides* Michx. and *V. brittonii* Porter ex Bickn. appear to occur only in the northwest corner of the state. *V. corymbosum* and *V. vacillans* occur throughout the state, *V. stamineum* appears limited to central and northern New Jersey, and *V. atrococcum* appears uncommon in northern New Jersey.

The diploid *V. vacillans* and the tetraploid *V. corymbosum* show considerable variation as the result of extensive hybridization (1). The latter is particularly variable. *V. vacillans* proved to be the best survey plant, for it is far more widely distributed in New Jersey than the other species and shows very distinct symptoms of stunt (Fig. 1-A,B,D,). Since it is a small plant only 6–18 in. tall (compared with 3–12 ft. for *V. corymbosum*), many plants could be seen at a glance. Clones extend over considerable areas by means of underground rhizomes, and stunt symptoms therefore often appeared in patches, making detection easier. Disease incidence in *V. vacillans* populations could be only approximated, for hybrids with *V. augustifolium* rarely show satisfactory symptoms, plants in burned-over areas show no easily detectable symptoms on the first growth, and the size of each clone cannot be readily determined.

Most of the survey locations were 1–5 miles from cultivated blueberry plantings. The northernmost locations were at least 70 miles from commercial plantings, and separated from them by open farmlands and metropolitan areas.

The survey for blueberry stunt in wild plants was based on visual symptoms. To be certain that those symptoms were actually caused by the stunt virus, transmission tests were made by budding and grafting, from representative wild bushes growing about 30 miles north of the principal commercial plantings, to healthy plants of the Jersey variety of the cultivated highbush blueberry. Transmission tests were also made from diseased wild bushes to *Vinca rosea* L. by means of dodder, *Cuscuta subinclusa* Dur. & Hilg.

Results.—Results of the transmission studies are summarized in Table 1. The virus transmitted to the cultivated Jersey variety from wild blueberry species


A
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appeared to be stunt so far as could be determined from symptoms. Grafted plants showed the characteristic chlorosis of the leaves, reduction in leaf size, stunting, and secondary branching.

The symptoms produced in *Vinca* by virus from wild blueberries appeared identical with those in *Vinca* infected by means of dodder from a known source of stunt in cultivated blueberry. Infected plants were also compared with stunt-infected *Vinca* plants in L. O. Kunkel's collection of yellows-type viruses at the Rockefeller Institute for Medical Research, New York, N. Y. In *Vinca* the virus was distinct from any other yellows-type virus. The leaves were pale green and marked with patches or transverse bands of dark green. Leaf size was not markedly reduced. Flowers of infected plants were smaller and fewer than those of healthy plants, but virescence did not occur.

The survey disclosed 17 affected plants of *V. atrococcum* at 5 locations, 57 plants of *V. corymbosum* at 20 locations, 55 plants of *V. stamineum* at 8 locations, and over 3000 plants of *V. vacillans* at 55 locations. Diseased plants of the last species were found at almost all locations surveyed. Locations in New Jersey where plants showed stunt symptoms are given in Fig. 2. Actual distribution and relative abundance of wild blueberries or the incidence of stunt are not shown, but the virus obviously is widespread in the wild blueberry population.

Discussion.—The fact that stunt is widespread in wild blueberries and coincides with large populations of the insect vector strongly suggests that the virus is indigenous to New Jersey. It is believed that stunt in wild blueberries was previously overlooked because of its supposed origin outside New Jersey, because of poor symptom expression in wild highbush blueberry, and because symptoms expressed by *V. vacillans* and other species of wild blueberries were not recognized.

The leafhopper vector, *Scaphytopius magdalensis*, was collected at 18 locations in New Jersey throughout the areas surveyed for stunt. Hutchinson (5) has shown that the vector will feed and reproduce on *V. corymbosum* and *V. vacillans* and that the vector strongly prefers a shaded environment to an open one. This latter tendency precludes long flights and reduces the possibility of wind dispersal. It seems improbable that stunt spread throughout the state in the 40 years during which blueberries have been cultivated, for spread throughout a small field may take as long as 5 years (3).

Since the entire wild blueberry population appears to be a reservoir of stunt virus, isolation of new blueberry plantings from presently cultivated fields is not enough to ensure freedom from stunt, as previously supposed. Leafhopper control, and possibly destruction of native blueberries in the vicinity of commercial plantings, should be practiced by all growers.—Crops Research Division, ARS, U.S.D.A., and New Jersey Agricutural Experiment Station, New Brunswick.

Fig. 2.—Map of New Jersey indicating locations where one or more wild species of *Vaccinium* have been observed with symptoms of blueberry stunt. The enclosed area shows the region in which highbush blueberries are grown commercially. The cross marks the location where stunt was first observed, and the 3 open circles indicate the only locations surveyed where no infected plants were observed.

Fig. 1.—Sympotoms produced by stunt virus: **A, B)** Diseased *V. vacillans* showing proliferation and reduced leaf size, compared with **C)** normal growth. **D)** Infected *V. vacillans* showing characteristic marginal chlorosis and cupping of leaves and lack of fruit, compared with **E)** normal growth. **F,G,H)** Twig and leaves of cultivated *V. corymbosum* var. Atlantic, showing chlorosis along leaf margins and between lateral veins, and cupping of leaves.

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THE IDENTITY AND HOST RELATIONS OF THE POTATO LATE-BREAKING VIRUS

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SUMMARY

Viruses obtained from late-breaking potatoes and Ladino clover with phyllody were compared in China aster, potato, Ladino clover, tomato, and *Nicotiana rustica* and found to be identical. Since this virus produced aster yellows symptoms in these hosts and was transmitted by the aster leafhopper it was considered to be a member of the aster yellows virus group. The virus was successfully transmitted to 15 of 24 plant species tested. Although the symptoms produced by this virus resembled those of the aster yellows virus in tomato, in Jimson weed they resembled those of the tomato big bud virus. Vine systems of the late-breaking isolate in potato closely resembled those described for purple-top wilt caused by either the aster yellows or tomato big bud viruses. Infection in potatoes in central Oregon occurred primarily during the first 2 weeks of July and the last 2 weeks of August. Hair-sprout was consistently associated with tubers from late-breaking potato plants, whereas tuber perpetuation of the disease was erratic. Incidence of the late-breaking disease in the field was closely correlated with the acreage of Ladino clover in the problem area.

Introduction.—In 1946 a disease of potatoes (*Solanum tuberosum* L.) new to Oregon appeared in most fields of Netted Gem, Burbank, and White Rose. A study of the disease by Milbrath and English (27) resulted in its characterization and application of the name "late-breaking virus disease" of potatoes. In 1952 this disease became epiphytotic in central Oregon, where 40–50% of the plants in several fields became infected, compared with a high of 3% in 1946. This paper reports the results of a study to determine the cause of this severe outbreak and to obtain additional basic information on this virus.

Review of the literature.—Table 1 summarizes the findings of various workers on viruses causing purple-top wilt and similar diseases of potato with respect to vectors, certain host plants, and the effects on potato. According to Milbrath and English (27), symptom expression of the late-breaking virus in potato suggested a relationship to the viruses that cause purple-top wilt (3), yellow-top (5), witches' broom (42), apical leaf-roll (3), haywire (10), and bunch-top (22). Epps (4) and Younkin (43) demonstrated conclusively that the aster yellows virus was a cause of potato purple-top in the United States. Folsom (5) concluded that yellow-top was closely related to aster yellows but Kunkel (18) was unable to transmit yellow-top with the aster leafhopper, *Macrosteles fascifrons* Stal, and showed that beet (*Beta vulgaris* L.) was susceptible to the virus though immune to aster yellows. MacLeod (22) reported that bunch-top virus resembled purple-top wilt in potatoes but more closely resembled tomato big bud virus in tomato (*Lycopersicon esculentum* Mill.). The tuber-perpetuated phase of the bunch-top virus in potato closely resembled the haywire virus disease described by Goss (10). Recently, Webb and Schultz (41) reported tomato big bud symptoms on tomato grafted with material from haywire-diseased potatoes. Though most reports of purple-top in the United States have been ascribed to the aster yellows virus, purple-top wilt in Washington was attributed to the tomato big bud virus by Menzies (24). In 1954 Norris (28) showed that purple-top wilt of potato in Australia was caused by the tomato big bud virus. More recently Helms (12) showed that at least one strain of alfalfa witches' broom virus is identical to the tomato big bud virus. She has also shown that this virus produces a "greening" of the flowers of China aster (*Calistephus chinensis* (L.) Nees), which indicates a possible close relationship to the aster yellows virus. The stolbur disease in central Europe and the Crimea was originally considered identical to the tomato big bud disease as described in Australia (26). In 1946 Sukhov and Vovk reported that the stolbur virus in Russia caused a disease of potatoes with symptoms identical to those caused by the aster yellows virus in the United States. According to Blattny et al (2) Sukhov and Vovk later differentiated the stolbur virus into "northern" and "southern" stolbur, differing from each other as well as from the Australian tomato big bud virus. Neither the tomato big bud virus nor northern stolbur virus caused a wilt of solanaceous plants, whereas the southern stolbur virus typically caused such a wilt. Northern stolbur was also known to occur north of the range of *Hyalesthes obsoletus* Sign., the vector for the southern stolbur virus. Blattny et al (2) found that

Table 1.—Findings of various workers on viruses causing purple-top wilt of potato and similar diseases

Virus and reference	Vector	Transmission to[a]			In potato		
		Aster	Legume	Potato	Tuber-perpetuated	Produces spindle sprouts	Produces vascular necrosis
Aster yellows, New York strain (16)	*M. fasciſrons*	+	−	−	−	−	−
Aster yellows, New York strain (4, 43)	*M. fasciſrons*	+	0	+	−	−	+
Aster yellows, California strain (6, 34, 35, 36)	*M. fasciſrons* & 21 other spp.	+	+	+	−	−	−
Potato apical leafroll (3)	*M. fasciſrons*	−	0	+	+	−	+
Potato witches' broom (8, 42)	*Ophiola flavopicta*	0	+	+	+	+	−
Tomato big bud (12, 13, 28)	*Orosius argentatus*	+	+	+	±	+	+
Southern tomato stolbur (2, 32, 37)	*Hyalesthes obsoletus* *Aphrodes bicinctus*	0	0	+	−	+	+
Northern tomato stolbur (2, 37, 38)	*Macrosteles* sp.?	0	0	+	−	+	−
Potato late-breaking (27, 29, 30)	*M. fasciſrons*	+	+	+	±	+	−
Beet curly top (9, 33)	*Circuliſer tenellus*	−	+	+	+	−	−
Cranberry false blossom (19)	*Euscelis striatulus*	−	−	+	0	0	0
Potato bunch-top (22)	None known	−	+	+	2	+	+
Potato haywire (10, 22, 41)	None known	0	0	+	+	−	+
Potato yellow top (5, 18)	None known	0	0	+	±	+	+
Potato rosette (14)	None known	0	0	+	+	−	+
Potato purple dwarf (31)	None known	0	0	+	+	−	+

[a] + = yes; − = no; 0 = no information; ± = erratic; 2 = through second generation.

Aphrodes bicinctus Schrk. was also a vector of the southern stolbur virus. Valenta (38) considered the northern stolbur virus identical to the potato witches' broom virus and suggested elimination of the name "northern stolbur" to avoid confusion. More recently Valenta (39) has isolated four strains of the stolbur virus as well as two other viruses, Crimean yellows and clover phyllody. In plant cross-protection tests he found that four strains of potato witches' broom virus did not protect against each other, the stolbur virus, or Crimean yellows virus. An interference phenomenon occurred in which symptoms of a potato witches' broom strain were completely suppressed by a strain of stolbur virus and by Crimean yellows. In these instances the witches' broom virus could not be reisolated. The clover phyllody virus did not protect against Crimean yellows in these experiments. Wright (42) reported that the virus from one of 13 sources of potato witches' broom from western Canada produced big bud symptoms on tomato. Fukushi et al (8) recently demonstrated that the leafhopper *Ophiola flavopicta* Ishihara was a vector of the potato witches' broom virus in Japan. Kunkel (19) indicated that symptoms of the cranberry false-blossom virus on a number of hosts suggested a close relationship to the tomato big bud virus. Strains of the beet curly top virus produce on potato a wide range of symptoms, many of which approach those of purple-top wilt and may have been confused with it (9, 25).

Materials and methods.—The late-breaking virus used in these studies was obtained from 3 sources: 1) naturally infected potatoes, 2) Ladino clover (*Trifolium repens* L.) with phyllody, and 3) viruliferous aster leafhoppers collected in Ladino clover and potato fields.

The aster leafhoppers used in these studies were colonized in the greenhouse from leafhoppers collected from Ladino clover in central Oregon and from a colony forwarded by Dr. J. H. Freitag, University of California, Berkeley.

Non-viruliferous aster leafhoppers were reared on Sacramento barley (*Hordeum vulgare* L.) or rye (*Secale cereale* L.), which are immune to the virus (1). Both male and female leafhoppers were caged on the plants with 8-in. cuboid cages.

The plants used in the transmission studies, grown in No. 10 cans, were inoculated in the 2–6-leaf stage, depending on the species.

For each transmission experiment non-viruliferous leafhoppers were transferred from rye or barley to a virus source plant for 3–7 days of acquisition feeding, and then transferred to other young rye or barley plants for 10 days before being placed on the test plants. Ten leaf hoppers were then placed on each test plant for 3–10 days, and transferred to successive test plants with an aspirator as long as they survived. After each transmission test the plant was dusted with 5% malathion, S-(1,2-dicarbethoxyethyl)-*O,O*-dimethyl dithiophosphate. After one day in the headhouse these plants were removed to a screened greenhouse that was fumigated weekly with tetraethyl pyrophosphate. Under these conditions there were no accidental transmissions to young aster plants maintained in the greenhouse as controls for the duration of the experiments.

In graft-transmission experiments, either side or whip grafts were used. The grafts were bound with Parafilm, and the plants kept in a moist chamber for about 6 days before being placed on the greenhouse bench.

Results.—*Comparison of viruses from potato and clover.*—Preliminary work (30) had shown that the late-breaking virus disease of potatoes was closely associated with a virus causing phyllody of Ladino clover. This relationship was tested by a comparison of symptoms produced in 5 species of plants by the

Table 2.—Comparison of symptoms induced[a] by viruses from late-breaking potatoes and Ladino clover with phyllody

Original source	Symptoms produced in:				
	Aster	Potato	Ladino clover	Tomato	*Nicotiana rustica*
LBV potato	AY[b]	LBV[c]	Phyllody	AY	AY
Phyllody Ladino	AY	LBV	Phyllody	AY	AY

[a] All inoculations were made by means of the aster leafhopper.
[b] AY = aster yellows virus symptoms.
[c] LBV = late-breaking virus symptoms.

virus from late-breaking potato plants and from Ladino clover with phyllody (Table 2). The late-breaking potatoes were grafted to *Nicotiana rustica* L., from which virus-free aster leafhoppers acquired the virus for transmission in these experiments. Aster leafhoppers were also used to transmit the virus found in the Ladino clover plants. At least 3 of 5 test plants of each species became infected with the virus from each source.

This test confirmed the earlier findings (30) that the virus causing the late-breaking disease of potatoes was identical to the virus causing a disease of Ladino clover characterized by severe stunting, proliferation of leaves, reddening of the leaflet margins, and phyllody. Since this virus produced symptoms of aster yellows in China aster (*Callistephus chinensis* (L.) Nees), tomato (*Lycopersicon esculentum* Mill.), and *N. rustica* L. and could be transmitted by the aster leafhopper, it was considered a member of the aster yellows virus group.

Host-range study.—1) *Susceptibility.*—Although the symptoms produced in aster and *N. rustica* by the late-breaking virus clearly illustrated a close relationship to aster yellows, transmission of the virus to Ladino clover was a possible point of differentiation. The only previously reported transmission of an aster yellows virus to legumes was by Frazier and Severin (6). Following a preliminary report of transmission of the late-breaking virus to clover by Raymer and Amen (30), Webb and Schultz (40) reported a phyllody condition of Ladino clover in Maine caused by transmission of an aster yellows virus to clover. More recently Halisky et al (11) reported the effects of the aster yellows virus on various *Trifolium* species in California.

For clarification of the relationship of the late-breaking isolate to other strains of the aster yellows virus and to similar viruses, a limited host-range study was made (Table 3). Insusceptible species were included in Table 3 only when leafhoppers from the same colony successfully transmitted the virus to aster plants in the same experiment.

2) *Symptoms.*—The symptoms induced by the late-breaking isolate on aster, dandelion, plantain, and tomato were identical to those described by Kunkel (15, 16, 17) for the aster yellows virus. The symptoms on celery resembled those caused by the California aster yellows (17). In the clovers, symptoms consisted of stunting, proliferation of shoots, reddening of the leaf margins, and phyllody of the inflorescence as recently described and pictured by Halisky et al (11). Jimson weed, though considered by Kunkel (15) to be immune to the New York aster yellows virus, is susceptible to both potato bunch-top and tomato big bud (13, 22). First symptoms of the late-breaking isolate of aster yellows in Jimson weed were yellowing and cessation of growth of the interveinal tissues of the youngest leaf while the midrib and lateral vein tissue continued to grow. This resulted in a retrorse curling of the leaf (Fig. 1-A). All subsequent leaves were much dwarfed, with very short petioles. The plants showed a bunchy-top condition within 5–6 weeks of inoculation (Fig. 1-B). No flowers developed on these plants. These symptoms resemble those produced in this host by the tomato big bud virus (13).

Common mullein, a perennial plant growing along the roadside in central Oregon, exhibited extreme stunting and yellowing when infected with the late-

Table 3.—Plants tested for susceptibility to the late-breaking isolate of the aster yellows virus from central Oregon

Plant tested	Scientific name	Number infected[a]
Alfalfa	*Medicago sativa* L.	0/18
Alsike clover	*Trifolium hybridum* L.	18/20
Aster	*Calistephus chinensis* (L.) Nees	92/117
Bean	*Phaseolus vulgaris* L.	0/36
Beet	*Beta vulgaris* L.	0/10
Celery	*Apium graveolens* L. var. *dulce* Pers.	7/39
Cowpea	*Vigna sinensis* Endl.	0/18
Crimson clover	*Trifolium incarnatum* L.	11/12
Dandelion	*Taraxacum officinale* Weber	5/10
Jimson weed	*Datura stramonium* L.	14/15
Ladino clover	*Trifolium repens* L.	21/30
Lima bean	*Phaseolus limnensis* Macf.	0/8
Mullein	*Verbascum thapsus* L.	1/5
Pepper	*Capsicum frutescens* L.	0/12
Plantain	*Plantago major* L.	4/6
Potato	*Solanum tuberosum* L.	73/218
Prickly lettuce	*Lactuca scariola* L.	11/11
Red clover	*Trifolium pratense* L.	12/20
Rustic tobacco	*Nicotiana rustica* L.	16/20
Scarlet runner bean	*Phaseolus coccineus* L.	0/6
Sow thistle	*Sonchus arvensis* L.	4/5
Soybean	*Glycine max* Merr.	0/12
Tomato	*Lycopersicon esculentum* Mill.	16/20
Zinnia	*Zinnia elegans* Jacq.	0/18

[a] Number infected, over the number inoculated. All transmissions were made with the aster leafhopper, *Macrosteles fascifrons* (Stal).

breaking virus in the greenhouse. No such plants were found in the field during these studies.

Kunkel (16) first demonstrated the susceptibility of *N. rustica* L. to the New York aster yellows virus. More recently (20) he distinguished between the New York and California aster yellows viruses on the basis of symptom expression in this host. The California strain produced compact clusters of twisted and dwarfed leaves on short petioles, whereas the New York strain produced thin shoots with long internodes. Freitag (7) recently demonstrated at least 3 strains of aster yellows virus on the basis of symptoms in *N. rustica* L. and *P. major* L. His "Tulelake" strain closely resembles the California aster yellows of Kunkel and Severin, whereas the "severe" strain resembles New York aster yellows.

In studies with the late-breaking virus, the predominant symptoms on *N. rustica* L. were those of the

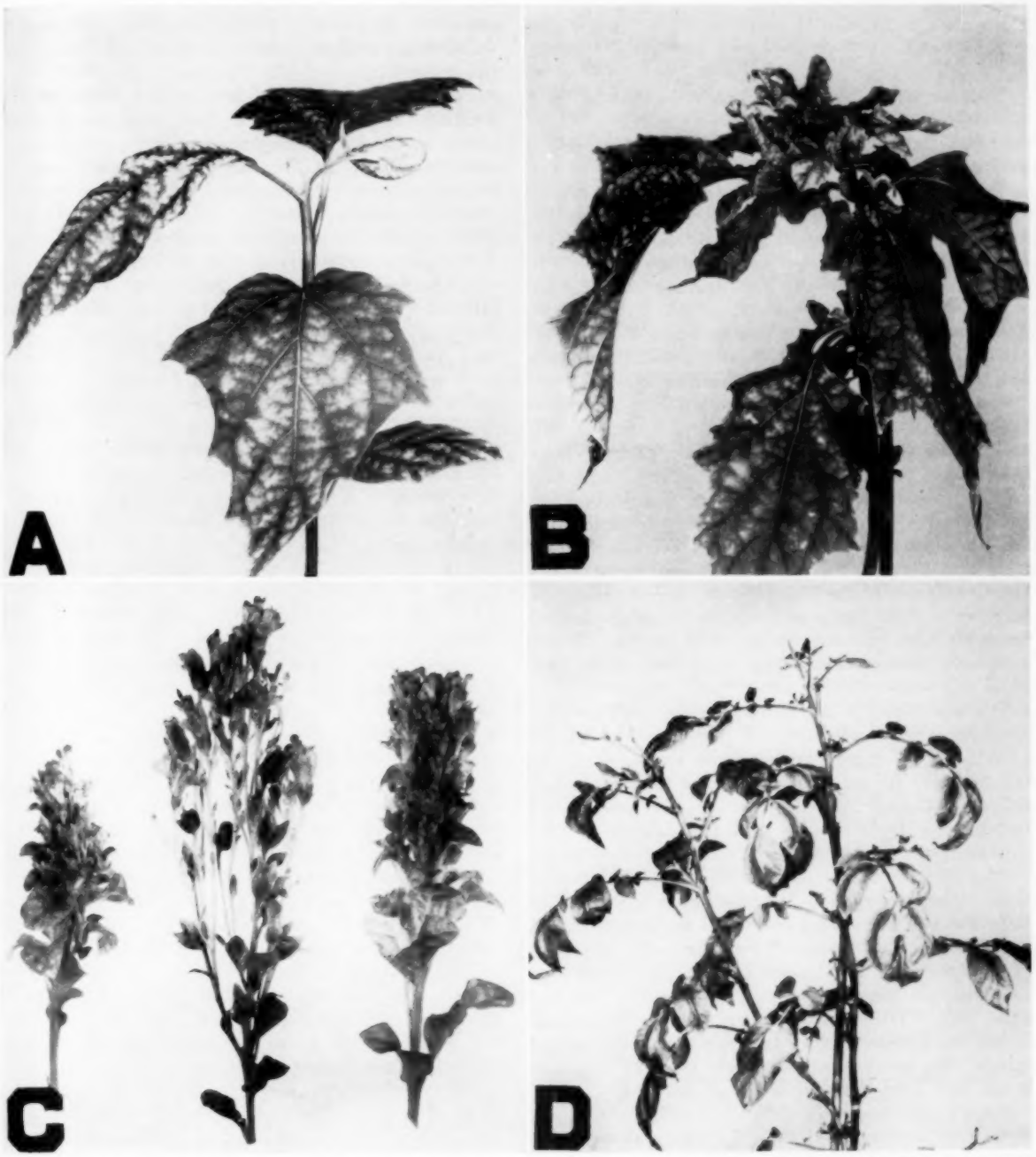

Fig. 1.—Effects of the late-breaking isolate of the aster yellows virus on various host plants. **A)** Early symptoms in Jimson weed. **B)** Late bunchy-top symptoms in Jimson weed. **C)** Left and right, usual symptoms in *Nicotiana rustica* L.; center, "severe" symptoms obtained occasionally. **D)** The late-breaking disease in potato variety Netted Gem.

Table 4.—Transmission of the late-breaking isolate of aster yellows virus to 10 potato varieties by the aster leafhopper

Variety	Number infected[a]
U.S.D.A. #41956	0/7
Burbank	4/8
Early Gem	2/5
Irish Cobbler	2/10
Katahdin	2/10
Kennebec	6/7
Netted Gem	29/115
Pontiac	1/5
Red Pontiac	2/5
White Rose	25/46

[a] Number infected, over the number inoculated.

"Tulelake" type. In some cases older plants developed symptoms of the "severe" type (Fig. 1-C).

Prickly lettuce, a common annual in the central Oregon region, was frequently found infected in the field. The distribution of naturally infected prickly lettuce plants in and near potato fields closely paralleled the incidence of the late-breaking virus disease in the potatoes. Severe stunting and chlorosis, together with proliferation of lateral shoots, were characteristic symptoms in prickly lettuce inoculated with the late-breaking virus in the greenhouse.

Kunkel (15) was unable to transmit New York aster yellows virus to tomato with the aster leafhopper but transmitted the disease to tomato by grafting from infected *N. rustica* L. The late-breaking virus was readily transmitted to tomato with the aster leafhopper. Symptoms developed in the manner described for Jimson weed but in addition there was a marked development of a purplish pigment in the veinal tissue and a strong epinasty of the older leaves. There was no enlargement of the calyx or proliferation of the lateral buds, as with the tomato big bud disease. These symptoms somewhat resemble those caused by beet curly top virus.

Ten varieties of potato were inoculated with the late-breaking virus by means of the aster leafhopper (Table 4). In these varieties, vine symptoms of the late-breaking isolate closely resemble those described for purple-top wilt caused by eastern aster yellows and tomato big bud viruses (21, 28). Most of the varieties expressed symptoms in the greenhouse, within 10 days to 3 weeks of inoculation, though the Netted Gem variety required 6–7 weeks. First indication of infection was an upward rolling of the apical leaves. Terminal growth stopped, a reddish purple marginal pigmentation of the upper leaflets developed, and tuberous swellings were finally produced in the leaf axils (Fig. 1-D). Pigmentation was most pronounced in the red varieties. Very few infected plants formed tubers. No necrosis of the lower stem or root was noted until after the tops of the plants died.

Time of infection.—In 1955 an experiment was performed to determine time of infection by the late-breaking virus under field conditions. Thirty young aster plants in No. 10 cans were transported to a potato field in central Oregon every 2 weeks from the middle of June to the first of September. The plants were placed at 25-ft intervals along the north and west borders of the field and exposed for two weeks. Then a new set of 30 plants was exposed and the previous set returned to the greenhouse at Corvallis, where they were dusted with malathion at weekly intervals until symptoms appeared. As symptoms developed they were recorded (Table 5) and the plants destroyed.

Potatoes in this field began to show late-breaking symptoms in the first week in August, indicating a delay in symptom expression of 3–4 weeks after infection.

Tuber perpetuation and hair-sprout.—Millbrath and English (27) demonstrated that tuber perpetuation of the late-breaking virus may occur for more than 2 generations but may be very erratic from season to season. Tuber perpetuation has not been demonstrated for purple-top wilt in the eastern United States (21) though it is a constant feature of apical leafroll (3) in Maine.

In the fall of 1953, 400 tubers from potato plants infected with late-breaking virus and the same number

Table 5.—Field exposure of aster plants to determine time of infection with aster yellows

Plant position	Periods of exposures 6/16 to 7/5	7/6 to 7/19	7/20 to 8/1	8/2 to 8/15	8/16 to 9/1
West border					
1	–	AYV[a]	–	0	0
2	0[b]	–	–	0	–
3	–	–	–	–	–
4	–	–	–	–	–
5	–	–	–	–	–
6	–	AYV	–	–	–
7	–	–	AYV	–	–
8	–	–	–	0	–
9	–	–	–	–	0
10	–	–	–	–	–
11	–	–	–	–	–
12	0	AYV	–	–	–
13	–	AYV	–	–	AYV
14	–	AYV	AYV	–	–
15	–	AYV	–	–	–
North border					
16	0	–	–	–	–
17	–	–	–	–	0
18	–	–	–	–	AYV
19	0	AYV	–	–	AYV
20	0	–	–	–	–
21	–	AYV	–	–	–
22	–	AYV	–	0	0
23	0	AYV	–	–	–
24	0	AYV	0	–	–
25	–	AYV	AYV	–	0
26	–	–	–	0	–
27	–	AYV	–	–	–
28	0	–	–	–	–
29	–	–	–	–	–
30	–	–	–	–	–
Totals[c]	0/22	13/30	3/29	0/25	3/25
Percentage AYV[a]	0%	43.3%	10.3%	0%	12%

[a] AYV = aster yellows virus.
[b] 0 = plants dead.
[c] Totals and per cent aster yellows virus were based on percentage of live plants.

from healthy plants were collected and stored at Corvallis. These tubers were allowed to sprout in early spring to check hair-sprout conditions. The sprouts were removed and the tubers later planted in the field in central Oregon. Hair-sprouts were produced by all tubers from late-breaking plants, and by no tubers from healthy plants. Tubers from late-breaking plants produced weak plants that later recovered and produced a nearly normal yield of potatoes. There was no increase of the late-breaking disease in potatoes adjacent to this plot, indicating that the disease had not spread from these plants.

In 1954 tubers were collected from 100 healthy and 100 diseased plants and stored through the winter. Hair-sprouts were produced by 42 of the 100 tubers from diseased plants, and by no tubers from healthy plants. Definite late-breaking symptoms developed in 3 of the plants from the 42 hair-sprout tubers, and in 4 of the plants from the 58 diseased tubers that produced no hair-sprouts. Tubers from the 7 diseased plants and from 30 randomly selected symptomless plants from the diseased block were collected in the fall of 1955 and planted in the greenhouse. Only the tubers from the 7 diseased plants produced plants with late-breaking symptoms. All 37 potato plants were tested for virus by grafting to tomato plants. Only tomato plants grafted with material from the 7 diseased potatoes developed symptoms of aster yellows. Thus, though the disease was definitely tuber-perpetuated in some cases, only a small percentage of the tubers from diseased plants reproduced the disease. Hair-sprouts were consistently associated with tubers from diseased plants, but some tubers with normal sprouts also reproduced the disease.

Field problem.—The epiphytotic of aster yellows virus in central Oregon potatoes during 1952–53 appears to have been due to the function of Ladino clover as a reservoir for the virus. Ladino clover was first grown in this area when a 50,000-acre irrigation project was completed, in 1946. Because of a good seed market, acreage of Ladino clover increased rapidly to a peak of over 21,000 acres by 1951. When the seed market collapsed, acreage dropped rapidly to a low of about 1100 acres in 1954. Very little late-breaking was seen before 1952, and incidence of the disease in potatoes up to that time was probably less than 10%, though no surveys were made. In 1952 over 50% of the potato plants in many fields became infected with late-breaking virus.

As the acreage of Ladino clover later decreased, the incidence of late-breaking in potatoes also decreased (Fig. 2). The lag between peak Ladino acreage (1951) and greatest disease incidence (1952) may represent the time necessary for spread of the virus within the clover or for the advent of a large leafhopper population. Aster leafhoppers were abundant in clover in the spring, and their movement to potato fields may have been triggered by clipping of the clover, usually done when potatoes were 3–6 in. tall. Incidence of aster yellows in the clover fields was not determined quantitatively, but a high proportion of leafhoppers obtained from these fields were carrying the aster yellows virus.

Discussion.—*Virus identity.*—From these experiments it is apparent that the late-breaking virus should be considered a member of the aster yellows virus group until a definitive study of strains is completed. This clarifies the broad relationships of the late-breaking virus, but the problem arises as to what constitutes the "aster yellows virus." Conceivably, strain variation could account for the differences found between apical leaf-roll, bunch-top, purple-top wilt, haywire, yellow-top, tomato big bud, northern and southern stolbur, and late-breaking and similar diseases of potato. The viruses that incite these diseases are all of the yellows type, and therefore not sap-transmissable. None have been purified or photographed with the electron microscope, nor has an anti-serum been prepared against any of them. Differentiation of these viruses is based entirely on symptoms, host range, and vector relationships. The symptoms in potato do not seem sufficiently distinct to justify retaining them as separate viruses on this basis alone. Some differences exist in host range, but information is insufficient to enable differentiation by this means. Even if all these viruses were compared on the same host species, the degree of difference in host range necessary to determine whether they should be considered as separate viruses rather than strains of existing viruses would be arbitrary and subjective. Vector differences within this group may be of little value as a criterion since Serverin (35) demonstrated that 22 species of leafhoppers and related insects are capable of transmitting a California strain of aster yellows. In other parts of the world only 6 different insects have been shown to be vectors of the viruses involved.

Despite its rather nebulous nature there are several ways of approaching the problem. First, a common host range could be selected and tested with the different viruses by the various workers throughout the world, or all these viruses could be collected in one location and compared under one set of conditions.

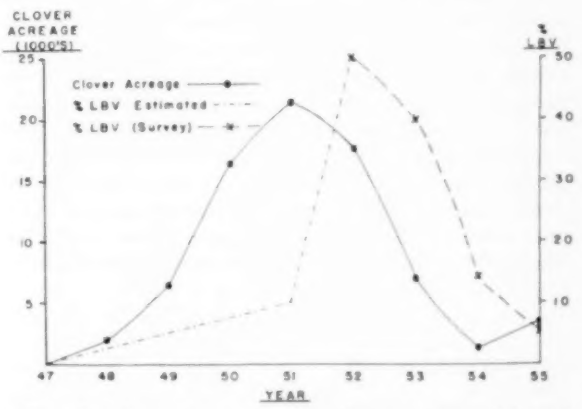

Fig. 2.—Comparison of Ladino clover acreage with incidence of the late-breaking disease of Netted Gem potatoes in Jefferson County, Oregon. The Ladino clover acreage figures were supplied by Mr. J. Ross, Extension Specialist in Farm Crops at Oregon State College.

Secondly, the different vectors could be tested for their ability to transmit these viruses. Maramorosch (23) recently presented evidence that *Macrosteles laevis* Ribaut is unable to transmit aster yellows although this species is morphologically very similar to *M. fascifrons* Stal, which can transmit the virus. This indicates that vector specificity may still be a useful criterion. Cross-protection in insects and plants as recently described by Kunkel (20), Freitag (7), and Valenta (39) is a very promising approach to differentiating yellows viruses for which other types of physical and chemical evidence are lacking.

Field problem.—As shown in Fig. 2, correlation was quite marked between incidence of late-breaking in potatoes and acreage of Ladino clover. Incidence of the virus in Ladino increased from year to year with increased acreage of clover, producing a large reservoir of aster yellows in the problem area. The other major crop grown in the area was grain. The aster leafhopper can multiply rapidly on grain and was abundant in these years. Thus, all biological variables were synchronized for development of the late-breaking epiphytotics in 1952 and 1953. This outbreak indicates that disease incidence is proportional to the reservoir of virus available as modified by fluctuations in the vector population and environment. It also illustrates the danger of long-term rotations with a perennial crop in which build-up of a virus may occur.

Control.—Control of this disease in potatoes presents a special problem since leafhoppers may infect several plants before being killed by insecticides applied to the crop. A more feasible approach may be to apply insecticides to clover fields before emergence of the potatoes. Spread from potato to potato within the field does not appear to be likely or important, for 2 reasons: 1) Aster leafhoppers were unable to recover the aster yellows virus directly from potatoes in several trials, though they could acquire the virus from *N. rustica* L. that had been grafted with material from late-breaking potatoes. The only report of direct recovery of aster yellows from potato is that of Severin (34) with a "long-winged" form of the aster leafhopper. 2) At least 11 days (1) must elapse from the time the insect feeds on a source plant until it is able to transmit the virus. In that time the leafhoppers probably leave the first potato field since potato is not a preferred host plant. Aster leafhoppers caged on potato plants usually start to die in 3 days, and all are dead in 10 days. Thus, the most likely source of virus is outside the potato field, and control measures must be designed accordingly.—Department of Botany and Plant Pathology, Oregon State College, Corvallis.

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INFLUENCE OF SOIL MOISTURE STRESSES ON THE DEVELOPMENT OF THE ROOT KNOT NEMATODE

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SUMMARY

With a split-root technique the root systems of Pearson and Rutgers tomato were held at 2 soil moisture stresses: field capacity (FC) and permanent wilting percentage (PWP). About 750 eggs of *Meloidogyne hapla* were added to each soil system. After 30 days, all roots at FC and one-half at PWP were collected and knot counts made per g fresh root weight. The remaining roots were held at FC for 30 additional days. On both tomato varieties, knot counts were significantly higher on roots grown at FC, and absent or few on roots at PWP. The roots at PWP 30 days and FC 30 days showed no significant increase in numbers of knots. It is concluded that the eggs of *M. hapla* hatch equally well at FC and PWP, but that the hatched nemas are unable to migrate at the higher soil moisture stress.

The responses of many plant species, and of their pathogens, to soil moisture stresses in the readily available range—field capacity (FC) to permanent wilting percentage (PWP)—have been shown to be distinctive. The phenotypic response of these reactions may be manifested by pronounced alteration of disease proneness of the hosts, variation in the parasitic capabilities of the pathogens, or both (2, 3, 6, 8).

The present investigation was undertaken to study the influence of soil moisture stresses in the readily available range on the activity of the root knot nematode (*Meloidogyne hapla* Chitwood) in soil, and its subsequent development in tomato (*Lycopersicon esculentum* L.).

Working with various solutions of organic and inorganic substances, Wallace (12) found that emergence of larvae of the sugar beet nematode (*Heterodera schachtii* Schmidt) was inhibited at concentrations producing osmotic pressures of about 15 atmospheres (atm). He concluded that "reduction in the rate of larval emergence in diffusate at higher concentrations . . . is due to an osmotic pressure exerted by dissolved salts."

In a similar study, Dropkin and Martin (4) found that NaCl solutions producing an osmotic pressure approaching PWP stress (15 atm) also inhibited the egg hatch of *Meloidogyne* spp. and *Heterodera rostochiensis* Wollenweber. Eggs of *M. arenaria* (Neal) Chitwood, however, developed normally in 0.3*M* NaCl (15 atms), though the larvae did not emerge until the eggs were removed to water. These results were interpreted as an indication that the hatchability of the nematode species studied was directly related to soil moisture stresses.

Using a less empirical approach to the problem, Wallace (11, 13) investigated the correlation between soil moisture content and soil aeration, and their influence on larval emergence of the sugar beet nematode. He found that reduction of the porosity of sand by the addition of small quantities of clay directly reduced larval emergence rates. The motility of nematodes declined as water was removed from pore spaces, but egg hatch was not directly affected by moisture stress. It was concluded that rate of emergence of larvae was related to pore space distribution rather than moisture stress.

Table 1.—Influnce of soil moisture stresses on development of the root knot nematode in tomato.

Trial[a]	Variety	Treatment	Knots/g fresh root weight[b]
A	Pearson	FC	21.9
		PWP	0.5
	Rutgers	FC	21.5
		PWP	0.7
B	Pearson	FC	24.9
		PWP	8.0
	Rutgers	FC	25.9
		PWP	5.6

[a] A = The root systems of each plant held at their respective FC and PWP stresses for 30 days from time of inoculation. B = FC root systems held for 30 days from time of inoculation. PWP root systems held for 30 days from time of inoculation and then irrigated to FC for 30 days.
[b] LSD at 5% = 10.8; at 1% = 14.3.

To date, a primary problem connected with studying soil moisture stresses within the readily available range has been an inability to maintain a soil system, supporting a growing plant, at points below FC. The most widely used valid technique for a study with growing plants has been to vary frequency of irrigations (3, 9). With this approach, soil moisture is extracted to a predetermined point between FC and PWP, after which the entire soil system is irrigated back to FC. This is a valuable technique in that it permits a duplication of field conditions, but it does not allow for the more basic consideration of the influence of continuous soil moisture stress of less than FC on the disease proneness of the host, or the parasitic capabilities of the pathogen.

Utilizing the principle that plant extraction of soil moisture is essentially stabilized at a point near PWP (10) the split-root technique of Hobbs and Bertramson (5) was modified to permit the present study to be conducted with living root systems near FC stress and at continuous PWP.

Two-compartmented non-porous containers, each compartment measuring 7×7×8 cm, were used. Twenty each of 6-week-old plants of the varieties Rutgers and Pearson were transplanted to the containers in the following manner: On the basis of orientation with the long axes of the stems, the root systems were divided into 2 equal portions. Cuts were then made at the basal ends of the stems, splitting them longitudinally for about 25 mm. This procedure made possible a greater separation of the 2 root portions of each plant.

One-half of the root system of each plant was then transplanted into each side of the 2-compartmented container. Each plant was thereby supported by 2 root systems; each dependent on a separate irrigation (Fig. 1).

The soil used was a Hagerstown Silt Loam, with a PWP of 10.1%, as determined by the use of dwarf sunflowers (7), and a moisture equivalent (1) of 24.5%. The moisture equivalent determination was through the courtesy of Dr. R. M. Hagan, Department of Irrigation of the University of California at Davis.

At the outset, 2 total weight calculations were made for the plant-soil-container systems. One determination gave the total weight with both sections irrigated to FC; the other allowed for one root system to be held near FC and the other at PWP. Weight requirements were periodically adjusted to allow for plant growth.

The soil systems of both compartments of the containers were held near FC by frequent irrigations until the roots had permeated the soil of the individual containers. Then one root system was allowed to extract to PWP while the other was maintained near FC stress.

With the soil supporting the individual root systems of each plant at their respective moisture stresses (FC and PWP), inoculations were made as follows: Inoculum was prepared by shredding fresh tomato roots heavily infected with *M. hapla*. With a 9-mm cork borer, a core was taken from the center of each individual compartment to a depth of 45 mm. Five g of inoculum, containing about 750 eggs, was tamped into each hole and covered with the original soil plug. To slow evaporation from the soil surface, the PWP side of each double container was covered with peat moss.

After inoculation, the plants were grown in a greenhouse at about 24–28°C. Irrigation of the PWP side of each double container was withheld, while the FC section was held near FC by frequent waterings to the predetermined weight.

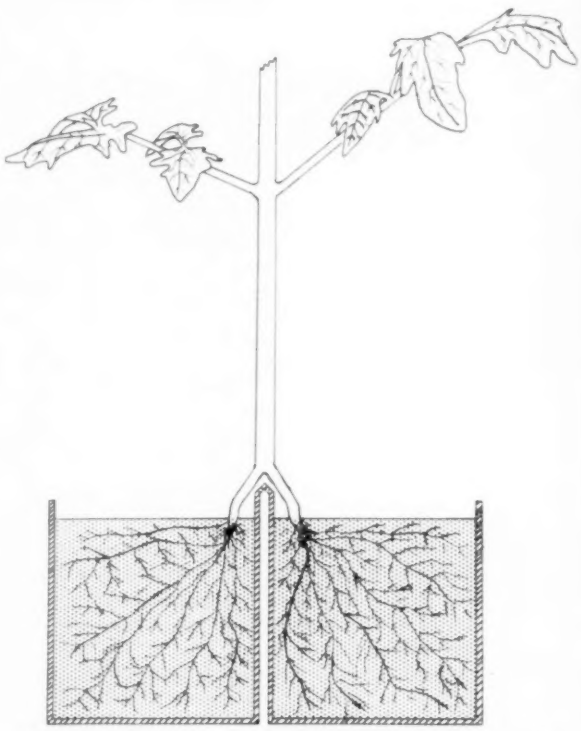

Fig. 1.—Split root technique used in investigation. One root system held at continuous PWP by withholding irrigations; contemporary root system maintained near FC by frequent irrigations.

Thirty days after inoculation, the first root knot incidence ratings were made. Each variety was divided into two 10-plant groups. Both root systems of the members of 1 group were harvested and rated (Trial A). With the second group, only the FC root systems were rated, and the intact PWP sections were irrigated to FC, held near this moisture stress for an additional 30 days, and then harvested and rated (Trial B).

Soil was removed from the root systems by first soaking, and then rinsing with a gentle flow of tap water. Disease incidence was recorded as the number of macroscopically discernible knots per g fresh weight of the individual root systems. All data were subjected to analysis of variance.

For both trials, the root systems of both varieties grown at FC from the time of inoculation contained a significantly greater number of knots than the root systems held at continuous PWP. Knot count for the root systems grown 30 days at PWP and then 30 days at FC (Trial B) showed an increase over the PWP group in Trial A, but not significantly so (Table 1).

Discussion.—As evidenced by the significantly higher number of knots on roots grown at FC, the influence of soil moisture stress at the extremes of the readily available range on development of *M. hapla* is very distinct. Contrary to Dropkin and Martin (4), however, high soil moisture stress did not inhibit egg hatch, but rather retarded migration of hatched nemas appreciably. This relation is evidenced by the fact that the PWP series of our Trial B showed no significant increase in knot count over the PWP series of Trial A, thereby illustrating that the bulk of the egg hatch had occurred during the initial 30-day PWP stress period.

In light of the present results and of Wallace's findings (11, 13) that moisture stress does not affect the hatchability of eggs of the cyst-forming species, *Heterodera schachtii*, but rather the motility of the hatched nematode, it is our conclusion that soil moisture in the readily available range plays an important role in the post-hatching phases of development of parasitic nematodes, though not in the developmental stages before and during hatching.

The split-root technique described, although not a complete answer to the problem of studying the influence of soil moisture stresses in the readily available range in the presence of intact and living root systems, should have many valuable applications in root disease investigations.—Department of Botany and Plant Pathology, The Pennsylvania State University, University Park.

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GALL INDUCTION BY MELOIDOGYNE INCOGNITA INCOGNITA BY SURFACE FEEDING AND FACTORS AFFECTING THE BEHAVIOR PATTERN OF THE SECOND-STAGE LARVAE

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Meloidogyne incognita incognita (Kofoid & White), the southern root knot nematode, has been the object of many scientific studies, yet few observations have been made on the living nematode (1, 4). In this investigation, in which live nematodes were observed with a microscope, we established a) the behavior pattern of the second-stage larvae, b) that certain sugars promote the movement of the larvae to the host root, and c) that gall formation frequently starts before the nematode has entered the host.

M. incognita incognita larvae were obtained by the following procedure. Egg sacs were shaken for 4 minutes in a test tube with a few ml of 10% Purex (a commercial bleach containing 5.25% NaOCl) (5); this broke up the sacs and surface-sterilized the eggs. The suspension was then poured into a tube containing 50 ml sterile water. The eggs that settled out in about 25 minutes were drawn off through a tap at the bottom of the tube, and transferred into 10 volumes of triple-strength Heller's solution (2) adjusted to pH 6.5 and containing 0.1 m*M* chelated iron (3). Three or four days later, larvae started to emerge from the eggs. With a dental pulp file, individual larvae were placed near the root tips of tomato (variety Kokomo) seedlings growing aseptically in Petri dishes. Each dish contained one plant and about 35 ml of 1% agar medium (White's minerals (6), adjusted to pH 6, containing 0.1 m*M* chelated iron); in some experiments this medium was supplemented with 0.1–0.5% cellobiose or sucrose. The seedlings were allowed to grow on these plates 1–2 days before any nematodes were added. These seedlings were originally obtained from seed germinated on filter paper in Petri dishes.

One larva was placed 3 mm from the tip of each seedling, and an examination was made immediately to ensure that only one larva and no eggs had been transferred. When placed 3 mm from tomato roots (variety Kokomo, seeds kindly supplied by ASGROW, of New Haven, Conn.) the larvae reached the root in about 45 minutes. Direct observation showed that the larvae were usually attracted to the terminal cm of the roots, though occasionally they were attracted to the second cm. This finding is in agreement with those of most investigators, though in direct disagreement with that of Wieser (7), who found root tips to be repellent to larvae. Wieser used excised roots to study attractiveness, and wounding effects may account for his findings. The inability of short excised root tips to grow in Wieser's experiments may also account for the supposed repellency of the tips, since attractiveness of the roots to larvae is related to the latter's growth. We found no indications that the root tips of intact tomato seedlings are repellent.

After reaching the root, the larva fed and moved along the root for 12–24 hours. During the latter part of this period the larva would locate an area of preference, where it would feed for another 12–24 hours. This area marked the future entry point into the root. In this period, the larva, immobile and closely appressed against the root, is almost indistinguishable from the root. Entrance into the host soon followed. More than 3 hours were taken for the larva to move its body into the root; the rest of the process was completed in less than half an hour.

It has been assumed that gall formation does not occur until the larva has entered the root, and frequently gall formation does not start until a day later. But numerous times gall formation was observed to begin before the nematode had completely entered the root; and in a few instances gall formation was seen without any nematode entrance (Fig. 1). In each of the latter cases a larva had been feeding at the site of the gall for some hours before moving away. Galls commonly continue to grow for at least 3 days

Fig. 1. Gall formed by a nematode in a tomato root without the nematode entering the root.

after the larva has entered the root, but nematode-free galls develop within a day and remain quite small. The above evidence supports previous reports that the larva secretes material(s) while feeding that induce gall formation.

M. incognita incognita larvae placed in the proximity of mustard (*Brassica nigra*) roots growing in plain agar medium were not attracted to the seedlings. Instead they wandered about aimlessly, sometimes passing over the roots but only rarely feeding; it appeared that they could not locate the roots. However, when sucrose or cellobiose was incorporated into the medium, the nematodes traveled directly to the roots, fed, and entered the host. Almost all the larvae entered the mustard roots growing on a medium containing sucrose or cellobiose; fewer than 4% entered the roots growing on plain medium. Gall formation was common among the former plants and absent among the latter. Possible explanations for the above action of sucrose and cellobiose are that a) sugars may induce mustard plants to excrete an attractant, b) the sugars counteract a substance(s) that prevents the nematode from locating the host, and c) the larvae utilize the sugars to overcome such a substance.—Department of Plant Pathology, University of Nebraska, Lincoln.

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CLONES OF RED CLOVER RESISTANT TO FOUR ISOLATES OF BEAN YELLOW MOSAIC VIRUS

Stephen Diachun and Lawrence Henson

The investigation reported is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director.

Bean yellow mosaic virus is probably the most common virus of red clover in Kentucky. Under field conditions, symptoms from plant to plant may vary considerably, because of genetic differences among the plants, even within a variety (1), and because of differences in the virus (3). Because differences among plants do occur, there is hope of selecting resistant plants for breeding stock. However, precautions must be taken, because a plant selected for resistance to one isolate of the virus may be susceptible to another isolate. For example, clone KyC101 was found to be resistant to 2 isolates but susceptible to 2 other isolates with which it was inoculated later. It is hoped that plants resistant to all strains of the virus can be found.

The purpose of this note is to report 1) ten clones of red clover resistant to 4 isolates of bean yellow mosaic virus, 2) reaction of 45 other clones to these isolates, 3) suggested possible uses for some of these clones.

Several hundred clones of red clover were made and maintained by cutting young shoots from vigorous healthy seedlings, mostly of the variety Kenland. The cuttings were kept in the greenhouse in tap water in beakers or glasses until roots developed, usually 10–14 days, and were then potted in steamed soil in clay pots. Usually there was no difficulty in establishing rooted cuttings in this simple manner, but soft rot occasionally destroyed the cuttings before roots developed. In hot summer weather, the unrooted cuttings were kept covered with wax paper or polyethylene plastic film.

Table 1 lists reactions of 55 selected clones to 4 distinct isolates of bean yellow mosaic virus. The

TABLE 1.—Symptom reaction of 55 selected clones of Kenland red clover to 4 isolates of bean yellow mosaic virus from red clover

Clone	Symptoms[a] induced by isolate			
	204-1	263	266	427
KyC4-1, KyC4-3, KyC4-18 KyC4-43, KyC7, KyC8-18 KyC19, KyC23, KyC36	M	M	M	M
KyC11, KyC25-2	M	M	M	0
KyC8-11, KyC16, KyC41	M	M	0	0
KyC8-17	M	0	M	0
KyC101	0	0	M	M
KyC18, KyC28-4	M	0	0	0
KyC17	M	Nec	M	M
KyC8-2	M	M	Nec	Nec
KyC4-9	M	M	Nec	0
KyC4-2	M & Nec	M	M	0
KyC5	M & Nec	M & Nec	Nec	M
KyC6, KyC6-13	Nec	Nec	Nec	Nec
KyC4-8, KyC25-18	Nec	Nec	Nec	0
KyC103	0	Nec	Nec	Nec
KyC8-5	Nec	Nec	M	M
KyC42, KyC104-1	Nec	Nec	0	0
KyC2, KyC6-6, KyC40-1, KyC61	Nec	0	0	0
KyC40-2, KyC71-8	Nec	M	M	M
KyC71	Nec	M	M & Nec	M
KyC14	Nec	M	M	0
KyC22, KyC40	Nec	M	0	0
KyC2-3	Nec	0	M	0
KyC25-7	Nec	0	0	M
KyC25-9	0	0	Nec	0
KyC25-19	0	0	0	Nec
KyC25-11, KyC28, KyC28-2, KyC28-3, KyC102, KyC104, KyC104-2, KyC104-5, KyC104-8, KyC105	0	0	0	0

[a] M = systemic mottle or vein yellowing, mild, moderate, or severe. Nec = local necrotic spotting with or without systemic necrosis, or systemic necrosis without observed local necrotic spots. M & Nec = combination of systemic mottle or vein yellowing and systemic vein necrosis. 0 = no symptoms.

original isolate used was virus isolate 204-1, considered typical of the virus common in naturally infected red clover plants in central Kentucky. Isolate 263 was detected in a mottled plant of clone KyC71-8, which develops necrotic-spotting with isolate 204-1, and isolate 427 in a mottled plant of Pennscott. Isolate 266 was recovered from a mottled plant of clone KyC101, in which isolate 204-1 does not cause symptoms. All 4 isolates were first observed in and isolated from naturally infected red clover.

Inoculations were made by rubbing 3 young carborundum-dusted leaves of each of 3 plants of each clone with a glass spatula dipped in inoculum prepared by crushing in .01*M* sodium sulfite young mottled leaves of infected plants of *Vicia faba* L., *Trifolium incarnatum* L., or *T. pratense* L. Inoculated leaves were briefly rinsed 2–5 minutes after inoculation, and some were dried with blotting paper. Clones that showed no symptoms were reinoculated at least twice. Plants were kept under observation for at least 6 weeks after inoculation. All inoculations were made in a greenhouse. In Table 1, the reactions are listed as M, systemic mottle or vein yellowing, mild, moderate, or severe; Nec, local necrotic-spotting with or without systemic necrosis, or systemic necrosis without observed local necrotic spots; M and Nec, combination of systemic mottle or vein yellowing and systemic vein necrosis; 0, no symptoms.

The table does not distinguish between mild, moderate, and severe symptoms that occurred. Also, the listing in Table 1 does not distinguish between necrotic-reacting clones that became readily invaded by the virus and those in which the virus was localized, but there are striking differences in this respect. For example, plants of clones KyC6, KyC14, and KyC71-8 were usually invaded systemically, whereas plants of KyC40 and KyC40-1 usually were not (2).

The table shows that in general the clones mottled by virus 204-1 were also mottled by the other virus isolates. Clones that were necrotic-spotting with virus 204-1 were also necrotic-spotting with other isolates. Clones that showed no symptoms when inoculated with isolate 204-1 did not develop symptoms when inoculated with the other isolates. Some very striking exceptions occurred.

At least 2 of the clones (KyC25-9 and KyC25-19) listed as necrotic were for a considerable time considered to belong to the symptomless group; it is quite possible that some of the clones listed as symptomless also belong to the necrotic group. Perhaps some clones that produce only a few or no local necrotic spots become systemically necrotic slowly and infrequently.

Because red clover can be propagated clonally without very much difficulty, and individual clones differ in their reaction to bean yellow mosaic virus, several practical uses resulted. Some necrotic-spotting clones have promise as breeding stocks for development of lines of red clover resistant to at least some strains of bean yellow mosaic virus (2). The resistant clones now reported may also be useful as breeding stock. Local-lesion clones were selected that are useful as assay plants (2). Two clones proved useful in protection tests for identification of strains or isolates of the virus (3). Progeny from crosses between selected clones provided information on the inheritance of reaction to the virus. Additional uses are developing. Some clones may be useful in maintaining cultures of virus isolates with reduced danger of contamination; for example, isolate 204-1 can be carried in clone KyC18, and isolate 263 in clone KyC22. Properly selected indicator clones might be used to detect new or different isolates of the virus. By observing symptoms that develop in groups of selected indicator clones set out in clover fields, it may be possible to estimate the prevalence of various isolates or strains of bean yellow mosaic virus in different locations. This would be much more convenient than the rather laborious procedure of making isolations and inoculations by hand. Other uses may be possible.—Department of Agronomy, University of Kentucky, Lexington.

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ISOLATION OF VICTOXININE FROM CULTURES OF HELMINTHOSPORIUM VICTORIAE

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The toxin of *Helminthosporium victoriae*, one of the most potent and most specific known, has been isolated and found to be a polypeptide complex (2). When subjected to mild alkali treatment the toxin is inactivated and broken down into two components (3): 1) a pentapeptide, which on hydrolysis yields amino acids tentatively identified as aspartic acid, glutamic acid, glycine, valine, and one of the leucines; and 2) a new base that has been given the trivial name "victoxinine." Victoxinine is a tricyclic secondary amine containing a single double bond. It has the empirical formula $C_{17}H_{29}NO$. The single oxygen atom appears to be in an ether linkage, and the single nitrogen atom, located close to the double bond, is in the form of a secondary amine. The labile linkage between victoxinine and the peptide appears to involve the amino groups of both victoxinine and the peptide. It is more stable than an electrostatic linkage since cleavage in the presence of sodium bicarbonate is not instantaneous. Unfortunately, further studies of the structure of the toxin are complicated by the difficulty of obtaining enough of the biologically active material to permit extensive chemical investigations. Many isolates of *H. victoriae* have been studied over the past several years. Most produced little or no

toxin in culture. When certain of these culture filtrates containing little or no toxin were examined, they were found to contain appreciable quantities of victoxinine. The finding that this material is present in a free state in metabolism solutions of poor or non-toxin-producing isolates of *H. victoriae* provides a readily available source of this substance for chemical and biological studies. The present paper outlines the procedure used in isolating free victoxinine directly from a culture filtrate and comparing its toxicity with the intact toxin.

Experimental methods and results.—Culture filtrate of *H. victoriae*, assaying less than 1:2000 (produced and assayed as previously (2) described), was evaporated from 50 to 5 liters *in vacuo* below 45°C in a large-capacity circulating evaporator. Methanol in equal volume was stirred into the solution, and the mixture was placed at 5°C for 48 hours. The precipitated material was then removed by filtration at 5°C, washed with cold 50% methanol, and discarded. The filtrate was concentrated *in vacuo* below 45°C to remove methanol. The resulting solution was clarified by stirring with 5 g Celite, cooled to 5°C, and filtered.

A saturated aqueous solution of mercuric chloride was added to the filtrate until precipitation was complete. The precipitate was removed by centrifugation, and washed by resuspending in 1*N* HCl and recentrifuging. The mercuric chloride complex was decomposed by suspending in 100 ml *N* HCl and saturating with hydrogen sulfide. The precipitated mercuric sulfide was removed by filtration, washed with 2×25 ml of *N* HCl and discarded. The combined filtrate and washings were adjusted to pH 8.6 with 10*N* NaOH, at which point the solution became opalescent from separation of victoxinine from solution.

This mixture was extracted three times with 50 ml diethyl ether to remove the victoxinine. The ether solution was evaporated *in vacuo*. The residue was dissolved in 15 ml *N* HCl. A saturated solution of mercuric chloride was added dropwise, causing a white crystalline precipitate to form. The precipitate was then removed by filtration, washed with 10 ml *N* HCl, suspended in 15 ml *N* HCl, and decomposed with hydrogen sulfide. After removal of the mercuric sulfide, the filtrate was evaporated to dryness *in vacuo* and dried *in vacuo* over solid sodium hydroxide. The viscous liquid resulting finally solidified and crystallized when treated with diethyl ether. The tan-colored, slightly hygroscopic crystals were removed by decanting the supernatant liquid, and were dissolved in 20 ml of absolute ethanol. Diethyl ether was slowly added to this solution, held at 30°C, until it became slightly opalescent. After remaining at room temperature for a few minutes, the opalescent solution was seeded with a crystal of pure victoxinine hydrochloride and scratched with a fine glass rod. Crystallization began at once in the form of fine colorless needles. After the mixture had remained overnight at 4°C to ensure comple ess of crystallization, the mother liquor was decanted and the crystals washed with cold ether and dried *in vacuo*. The yield was 220 mg. The 50 liters of original culture filtrate, which assayed less than 1:2000, would have produced less than 5 mg of the intact toxin.

After recrystallization from ethanol ether the hydrochloride melted at 172–173°C, and no depression of this melting point occurred when a sample was mixed with pure victoxinine hydrochloride previously isolated from the Helminthosporium toxin. The two samples showed identical infrared absorption spectra.

Toxicity of victoxinine.—Tested as previously described (2), victoxinine hydrochloride completely inhibited the growth of oat seedlings at a concentration of 2.5×10^{-4} molar (75 μg/ml), a toxicity of the same order as that of fusaric acid and lycomarasmin. The Helminthosporium toxin is about 7500 times as toxic as victoxinine on a weight basis for susceptible oat species. Both Helminthosporium toxin-resistant (Clinton) and Helminthosporium toxin-susceptible (Park) oat varieties were equally sensitive to victoxinine. When 0.1% ethanol was added to the test solutions the toxicity to both varieties was increased tenfold. Controls showed no toxicity until the concentration of ethanol alone was raised to 1%. Gäumann (1) showed a similar synergistic effect between alcohol and fusaric acid in tomato cuttings.

The finding that victoxinine may be isolated directly from culture filtrates of poor or non-toxin-producing isolates of *H. victoriae* and readily crystallized as the hydrochloride provides a readily available source of this new toxic base for chemical and biological studies. —The Rockefeller Institute, New York City.

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COLLETOTIN, A TOXIN PRODUCED BY COLLETOTRICHUM FUSCUM

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Digitalis lanata and *D. purpurea* plants, artificially inoculated with a spore suspension of *Colletotrichum fuscum* Laub., show a sudden foliar wilt after incubation for 6–7 days. Initial symptom expression—a limited number of water-soaked leaf lesions and wilted leaf edges—occurs after 5 days, and intense wilt of the entire leaf ensues from the sixth day after inoculation. Complete collapse of all leaves follows within

24 hours, and the plants are dead by the 8th or 9th day. This series of events suggests that *C. fuscum* might be producing a toxin.

To test this possibility a culture (Von Arx strain) of the pathogen obtained at Baarn, Netherlands, and 2 sporulating cultures isolated by the author from seedlings of *D. lanata* and *purpurea* were examined for toxin production. A modified Richards solution (100-ml quantities) was inoculated with spore suspensions of the above cultures and incubated at 27°C on a rotary shaker. Toxin production was detected with a bioassay developed by Gäumann and Jaag (1). In this assay a shoot bearing the 4 terminal leaves of a tomato seedling is excised and the stem end of the cut tomato shoot is placed in a vial containing the culture filtrate to be tested. Activity of the toxin is measured by intensity of symptoms.

Initial evidence of toxin production from culture filtrates of *C. fuscum* was usually apparent on the eleventh day of incubation. Effects induced on tomato foliage became progressively more intense through the 17th and 18th days. Concomitant with an increase in toxin production was a rise in pH—from 4.5, noted 24 hours after inoculation, to 6.8–7.0, at the end of the 17–18th day of incubation. During this period the culture filtrate characteristically became progressively more yellow. Each of the three isolates of *C. fuscum* tested was capable of producing the toxin.

Symptoms induced on tomato foliage are shown in Fig. 1. Under a microscope, foliage thus affected revealed numerous sunken or pitted areas. The epidermal cells in these areas appeared relatively normal, whereas the palisade cells had collapsed. The vascular tissues within the sunken regions were a light brown. These symptoms were evident 24 hours after the tomato shoots had been permitted to absorb the toxin for the standard 6-hour period. The earliest symptom was a loss of turgor by the leaves, which was regularly regained despite the intense necrosis sustained. The most intense "spotting" always occurred on the lowermost leaf of the shoot, which was the most fully expanded and probably exerted the greatest transpirational pull on the toxin-containing reservoir.

The effect of the toxin was quite different on *Digitalis* from that on tomato. The excised *Digitalis* leaves, with petiole base submerged in solutions containing 2500 μg/ml of the partially purified toxin, demonstrated reactions to the toxin after 3 days. The syndrome included a leaf-edge wilt, paling of the chlorophyll, first at the wilted periphery and then throughout the leaf, and finally a complete and permanent loss of leaf turgor. These symptoms were similar to those observed following artificial inoculation of the intact plant by a spore suspension. After the *Digitalis* leaves had been exposed 6 days to the toxin-containing solutions the entire syndrome was apparent at concentrations as low as 17 μg/ml of the partially purified toxin. Further, symptom expression on leaves was much more pronounced on *D. lanata* than on *D. purpurea*. Significantly, the latter is more resistant to *C. fuscum*.

Efforts were made to purify the toxin. The active principle in the culture filtrate (that which induced "spotting" of tomato foliage) was precipitated with acetone. A 1:1 (v/v) acetone-culture filtrate mixture resulted in an extremely heavy precipitate, which usually proved to be inactive and was therefore discarded. Successive additions of acetone to the filtrate, up to 6:1 (v/v), yielded the active substance; however, most of the activity was obtained from the 2:1 and 3:1 additions.

Lyophilized culture filtrates, before purification with acetone, showed profound spotting activity on tomato foliage at about 200 μg/ml. Upon extraction of the culture filtrate with acetone, the resultant amorphous precipitate induced leaf lesions regularly at concentrations of 60 μg/ml, and occasionally at 30 μg/ml.

In extracting large quantities of culture filtrate, the acetone precipitate was filtered through a 1-cm layer of celite at the base of a Buchner funnel. The celite was washed 3 times with ion-free water to remove the extremely water-soluble toxin principle. Removal of the water by lyophilization left a white amorphous powder.

Physical characteristics of the toxin principle were as follows: it was insoluble in organic solvents such as methanol, ethanol, ether, chloroform, methyl cellosolve, and pyridine. It resisted deactivation when reacted with cold acid and alkali for periods up to 1 hour and was not destroyed when boiled for a similar period in water. Finally its activity was not

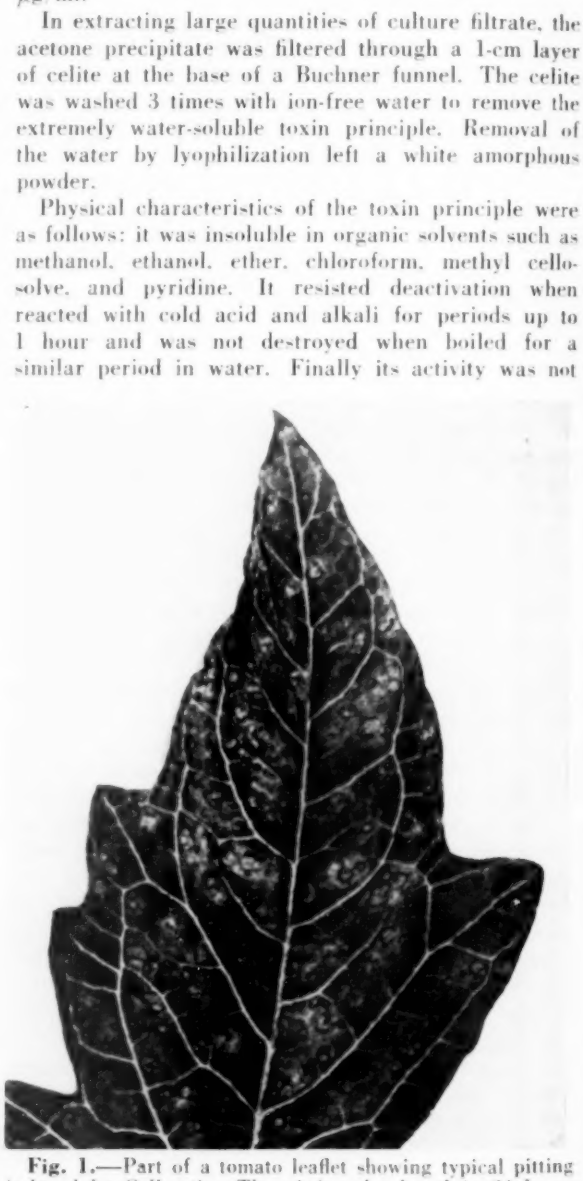

Fig. 1.—Part of a tomato leaflet showing typical pitting induced by Colletotin. The pitting developed in 24 hours and is the result of a 6-hour exposure to the toxin.

adsorbed by either cationic or anionic exchange resins, IR-50 and IR4-B; however, it was adsorbed by Franconite, a kaolin-type clay.

Chromatographic separation of the toxin from other components of the amorphous precipitate was unsuccessful in n-butanol–acetic-acid–water, n-propanol–water, and phenol–water systems. Two fluorescent fractions and a ninhydrin-positive fraction migrated in these systems, but on elution from the paper were found to be inactive. In all instances toxin activity was detected only at the origin of the chromatogram.

Hydrolysis of the toxin (amorphous powder containing the toxin) with 6*N* HCl for 1 hour at 100°C yielded a complex that gave an exceedingly strong positive reaction for reducing sugars with Bertrand's solution. Chromatogramming this hydrolysate and developing with aniline phthalate revealed the presence of glucose, galactose, and mannose; the movement of amino acids was detected with a ninhydrin spray. These same sugars were detected following a mild hydrolysis of the toxin complex with 0.5*N* HNO_3; however, this hydrolysate failed to liberate amino acids. Of particular importance here is the fact that both the strong and mild hydrolyses destroyed the activity of the toxin.

A solution containing the toxin complex gave a positive reaction for polysaccharides when reacted with anthrone reagent, and the presence of peptides was established upon the addition of folin reagent.

Finally, dialysis of the toxin-containing precipitate indicated that the active fraction was not dialyzable. It is this fraction, which causes the "spotting" of tomato foliage, that I have named "Colletotin." Further, reacting the solutions on both sides of the membrane with folin and anthrone reagents indicated that the polysaccharide moiety remained essentially inside the dialysis system, whereas much of the peptide fraction was dialyzable.

From the foregoing chemical and physical tests it is concluded that the active fraction of crude Colletotin contains both a polysaccharide and peptide fraction. It is also possible that the integrity of the polysaccharide entity is essential for the maintainence of activity.—Institute for Special Botany, Swiss Federal Institute of Technology, Zurich, Switzerland.

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LOSS CAUSED BY LATE INFECTION OF CANTALOUPES BY THE CURLY TOP VIRUS

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It has long been known that curly top virus will infect cantaloupe and certain other cucurbits, although the symptoms are erratic and unreliable (4). Cantaloupe plants infected with curly top virus are reduced in size and yield; plants infected while small are often severely affected, and plants larger at infection are usually affected less (2, 3). This has led growers generally to believe that late infection by curly top does not seriously damage melon crops.

The curly top virus is composed of an undetermined number of strains. Giddings (1) reported that 2 of his strains did not cause recognizable symptoms in melon plants. Hills and Taylor (3) worked with 3 strains, one relatively severe in cantaloupe.

The rate of curly top infection in good indicator plants, e.g. tomato, was high in most of the desert melon-growing areas in the Colorado desert in the spring of 1958. During this period the beet leafhopper, *Circulifer tenellus* (Baker), was present in high population. The cantaloupe crop was very poor that season in all the Colorado Desert districts except Yuma. Vines were stunted, yield was low, and the melons were of poor quality. Curly top infection of the melon plants was suspected but proof was lacking since there were no checks. Practically all the melon plants in the affected districts showed the same poor condition.

In the spring of 1959 beet leafhopper populations and curly top infection were low in the Imperial Valley and very low in the Palo Verde Valley. The practical absence of curly top coincided with an excellent crop of melons and made possible field comparisons of experimental early and late infections without interference from natural curly top infection. Plots were set up about 3 miles south of Blythe, Calif., in a cantaloupe field (variety 450) belonging to Wilco Produce Co. In certain rows every third hill was inoculated with certain viruses including curly top. Throughout the season this field remained essentially free of pests other than powdery mildew.

On March 23, 1959, ten beet leafhoppers that had fed for 3 days on a sugar beet plant infected with curly top virus were confined by cloth cages to each of 46 cantaloupe plants. The plants then had 1 to 2 true leaves. Of the 46 plants, 22 developed curly top symptoms covering a considerable range. Most were severely stunted, stopped growing at 6–20 leaves, and bore no fruit. By picking time these plants were usually overgrown by healthy plants, and were dead. A few developed 30–45 leaves and bore small melons, and one plant of 200+ leaves, bore a normal crop of three 36's (size: 36 per crate), then showed curly top symptoms and ripened the melons early.

On April 15th 16 plants were each inoculated with 20 beet leafhoppers that had fed 3 days on a sugar beet plant infected with a strain of curly top virus obtained in 1958 from cantaloupe in the Imperial Valley. This melon strain of the curly top virus produces very mild symptoms in sugar beet. The melon plants had 5–49 true leaves (av. 15.1) at inoculation. Of the 16 plants, 12 developed recognizable symptoms of curly top. They were more or less stunted, the leaves were gray-green, the tips of the runners were often chlorotic and later somewhat necrotic, and there was some necrosis of basal leaves. Plants that developed symptoms had averaged 12.3 true leaves at inoculation. Most of them stopped growing at 40–50

Table 1.—Crates of cantaloupe per acre from healthy plants and plants with symptoms of late and early curly top infection from inoculations made March 25 and April 15, 1959.

	Melon Size[a]				
Plants	Smaller than 45	45	36	27	Total (45 & larger)
Healthy	2	19	231	99	349
Late curly top	25	133	21	0	154
Early curly top	0	10	13	0	23

[a] Cantaloupe size is shown by the number packed per crate. Thus, 36 is larger than 45.

leaves, though some grew to various sizes up to the normal 250+ leaves. All the late-infected plants bore some fruit, most of it small, poorly netted, elongate, and low in sugar. One full-sized plant bore three 45's which ripened several days before the first pick. On June 2 the melons on all the plants in the plots were measured and counted. Table 1 shows production, on a per-acre basis, for healthy plants and for plants with late or early curly top infection. The figures show a considerable decrease in yield from late curly top infection, partly from smaller size. Actual shipments would be further reduced by poor shape, sunburn, cracking, and picking damage. A field infected with curly top would normally show a combination of early and late infection, with possibly some escapes, so that there can be a considerable range of production from such a field.

Some fields in 1958 had so much early curly top infection that the stand was lost, but in most the symptoms were stunted plants, small size, premature ripening with low sugar, and attendant troubles such as sunburn. It now seems quite certain that the crop failure in 1958 was caused by curly top infection spread by the large beet leafhopper population, and that late infection of melons by curly top virus can be more serious that has been generally recognized. Since the trouble in 1958 was generally called crown blight it is obvious that late curly top infection can contribute to the appearance of at least a part of that syndrome.

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This book contains papers given at a symposium on July 5, 1958, at the Institute of Microbiology in honor of Dr. Selman A. Waksman. The titles and authors are: Microbial Biochemistry and its Development, J. H. Quastel; Antibiotics—a New Field for Microbiological Research and Perspectives for the Future, H. Boyd Woodruff; Episodes in Immunochemistry, Michael Heidelberger; Bacterial Classification—Problems and Developments, S. T. Cowan; Some Contributions of Genetics to Microbiology, Vernon Bryson; Aspects of Russian Microbiology, George K. Skriabin; and Microbiology—Yesterday and Today, Selman A. Waksman. In the words of Dr. Waksman "The microbiologists of yesterday were largely concerned with a search for organisms responsible for known processes." They were primarily biologists, interested in the "broad biological activities rather than in specific biochemical reactions." The microbiologists of today, on the other hand, are primarily concerned with the mechanisms and reactions of specific organisms, and they utilize the quantitative methods and biochemical approach of the biochemist.

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This book was prepared at the Rubber Research Institute, Kuala Lumpur, Malaya, as a semi-technical manual for the practical rubber grower and advisory officers. It describes the symptoms, causes, spread, and prevention of 41 parasitic and nonparasitic diseases of the Para rubber tree, "hevea." The signs and symptoms of each disease are depicted in outstanding water colors by Mr. Hoh Choo Chuan. Common names of diseases are given in English and Malay.

MARTIN, HUBERT. 1959. **The scientific principles of crop protection.** 4th edition. Edward Arnold Ltd. London. 359 p. Price $12.50.

This is a revised and enlarged edition of the well-known "Scientific principles of plant protection," first published in 1928 and last revised in 1940. The 4th edition follows the plan of the 3rd. Several chapters have been materially expanded, and a new chapter added: "The measurement and mechanism of toxicity." As might be expected, much of the new information pertains to the large number of new pesticides introduced within the past 10 years and to the rapidly expanding literature on the mechanisms of toxic action.